101. *The Application of the Method of Molecular Rotation Differences to Steroids. Part II. Unsaturated Sterols and their Derivatives.*

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The data available in the literature on the optical rotatory powers of unsaturated sterols and their derivatives have been correlated by critical examination of their molecular rotation differences (M.R.D.'s). It is shown that the M.R.D's (Δ values) between unsaturated sterols and their fully hydrogenated analogues are characteristic of the position and type of unsaturation in any part of the molecule. A number of sterols previously considered to be impure (Part I; Barton, *J.*, 1945, 813) also show anomalies when examined by the

procedure developed in this paper: in general the conclusions reached in Part I are confirmed, wherever this is possible, by the data discussed here.

Revised or new formulations are proposed for ergosterols B_1, B_2, and D, and dihydroergosterol II is suggested to be identical with α-dihydroergosterol. Critical examination of both the physical and chemical evidence concerning β-dihydrofucosterol, dihydroergosterol III, ergosterols E and F, β-ergostadiene, and the $\Delta^{6,8(14)}$- and $\Delta^{7,9}$-cholestadienes shows that these substances are not homogeneous.

The development of a further rule correlating the optical rotatory powers of dienes and αβ-unsaturated ketones in the steroid series is described.

THE chemistry peculiar to the steroid group as a whole dictates two general methods of approach in effecting correlation between optical rotatory power and structure. The first, which has already been developed in Part I (Barton, *loc. cit.*), deals with the changes in optical rotatory power produced by transformations at the 3 position, and is particularly useful in characterising and differentiating naturally occurring sterols and triterpenoids (compare Barton and Jones, *J.*, 1944, 659). The second method, which is valuable in dealing with the more detailed chemistry of the group and in characterising conjugated dienes and substances of high rotatory power, is developed in this communication. It depends upon the differences in molecular rotation observed when unsaturated compounds are compared with their corresponding saturated analogues (in this paper with rings A and B in the *trans* position). The relationship need only be a formal one and it is not necessary that these transformations be effected by direct chemical methods.

The procedure is conveniently illustrated by the data listed in Table I. The term " Δ value," which is used throughout this paper, signifies the difference observed when the molecular rotation of the unsaturated substance is subtracted from the molecular rotation of its saturated analogue. Thus the Δ value of cholesterol is the molecular rotation of cholestanol less that for cholesterol itself, while for ketones, like Δ^4-cholestenone, it is defined as the value for cholestane less that for the ketone. Where the molecular rotation of a substance has been quoted before, it is not repeated here and the Δ values in Table I can easily be checked by reference to Part I. It will be noted that the Δ value for any sterol is highly characteristic of the type of nuclear unsaturation in that compound and that it varies to some extent as the C_3 hydroxyl is acylated. These facts permit a facile evaluation of double bond position. There are, therefore, three general methods of characterising the nuclear unsaturation in a sterol: the M.R.D.'s (Δ values) on acylation and on reduction, which are reasonably precise, and the absolute magnitudes of the rotatory powers, which are good working guides.

TABLE I.

Substance type.	Position of nuclear double bond (⌐).	Sterols. No. of examples.	Sterols. A.M.* of Δ values.	Sterols. Limits of variation from A.M.	Acetates. No. of examples.	Acetates. A.M. of Δ values.	Acetates. Limits of variation from A.M.	Benzoates.† No. of examples.	Benzoates.† A.M. of Δ values.	Benzoates.† Limits of variation from A.M.	Refs.
Δ^5-Stenols	5 : 6	6	+251‡	+10 to −9	6	+243	+27 to −18	6	+173	+16 to −27	1
γ-Stenols	7 : 8	2	+ 77	+16 to −17	2	+ 57	+3 to −4	1	+ 64	—	1
δ-Stenols	8 : 9	1	+ 48	—	1	± 0	—	—	—	—	1
α-Stenols	8 : 14	4	+ 9	+7 to −8	4	+ 14	+9 to −9	2	+ 53	+6 to −6	1
ε-Stenols	9 : 11	1	−100	—	1	− 77	—	1	−103	—	1
β-Stenols	14 : 15	3	− 24	+12 to −7	3	− 20	+16 to −14	1	− 59	—	1
7-Dehydro-Δ^5-stenols	5 : 6 and 7 : 8	5	+538	+40 to −40	3	+384	+21 to −27	2	+370	+13 to −13	1

* Arithmetic mean.

† Dinitrobenzoates are included under this heading as their molecular rotations are almost identical with those of the corresponding benzoates (see Reference 1).

‡ All rotations in this paper are in $CHCl_3$ for the Na_D line unless specified to the contrary.

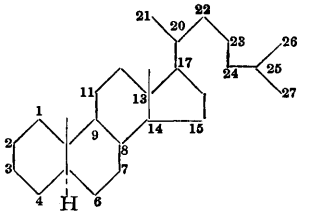
Table II lists a number of sterols whose Δ values are anomalous. The purity or assigned structures of bombicesterol, spongillasterol and Heilbron's Sterol B have already been questioned in Part I, and it is consequently not surprising that they show this anomalous behaviour when re-examined by the method developed here. Fucosterol is considered to be $\Delta^{5,24(28)}$-stigmastadienol (MacPhilllamy, *J. Amer. Chem. Soc.*, 1942, **64**, 1732) and its dihydro-derivatives can now be characterised. In agreement with Coffey, Heilbron, and Spring (*J.*, 1936, 738) α-dihydrofucosterol clearly possesses a 5 : 6-double bond and is thus identical with β-sitosterol. On the other hand β-dihydrofucosterol also appears to be substantially a 5 : 6 stenol; since both isomers are said to yield stigmastanol on complete hydrogenation (Heilbron *et al.*, *J.*, 1935, 1205), it is

TABLE II.

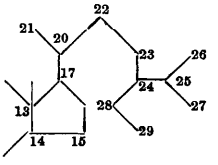
Substance.	Suggested position of ⌐: literature.	Suggested position of ⌐: this paper.	$[M]_D$ Sterol etc. Sterol.	$[M]_D$ Sterol etc. Acetate.	$[M]_D$ Stanol etc. Stanol.	$[M]_D$ Stanol etc. Acetate.	Δ values. Sterol.	Δ values. Acetate.	Refs.
Bombicesterol	—	Impure	−124	−188	− 43	+ 43	+ 81	+231	1
α-Dihydrofucosterol	5 : 6	5 : 6	−157	−201	+100	+ 69	+257	+270	1, 2
β-Dihydrofucosterol	—	Mixture	−124	−178	+100	+ 69	+224	+247	1, 2
Spongillasterol	22 : 23	5 : 6 with some impurity	−174	−219	+100	+ 69	+274	+288	1
Sterol B	24 : 28	Mixture	−107	−128	+ 25	− 37	+132	+ 91	1

likely that the β-compound is a mixture of the α-derivative with some stigmastanol. This conclusion is supported by the anomalous optical behaviour of *epi*-β-dihydrofucosterol (compare Callow and Strain, *Proc. Roy. Soc.*, 1936, A, **157**, 194), which can be interpreted on similar grounds.

In the same way as with nuclear unsaturation, the positions of double bonds in the side chains of sterols can be readily characterised. Table III lists sterols which may be compared in this way. Reduction of the $C_{22:23}$ bond is accompanied by an increase of approximately 61 in molecular rotation. There is one exception, ergosterol peroxide, to this generalisation and the most reasonable explanation of the discrepancy is that the rotation of this substance has been incorrectly recorded.



(I.)
Cholestane series.



(II.)
Stigmastane series.

TABLE III.

Substance.	Position of formally reduced ⊨.	Δ values. Sterols.	Acetates.	Dinitrobenzoates.*	A.M. of all values.	Limits of variation from A.M.	Refs.
Brassicasterol	22 : 23	+ 62	+ 89	+68			1
Ergosterol	22 : 23	+ 81	+ 42	—			1
$\Delta^{6,22}$-Ergostadien-3(β) : 5 : 6-triol	22 : 23	+ 60	—	—	+61	+28 to −19	3, 4, 5
Poriferasterol	22 : 23	+ 53	+ 49	+48			1
Stigmasterol	22 : 23	+ 49	+ 63	+72			1
24 : 25-Dehydrocholesterol	24 : 25	− 5	—	− 1			1, 6
Zymosterol	24 : 25	+ 1	− 8	+11	+ 1	+10 to −8	1, 6
epi-Zymosterol	24 : 25	+ 5	—	—			1, 6
Fucosterol	24 : 28	+ 20	+ 26	+10	+19	+7 to −9	1
Anomalous.							
Ergosterol peroxide	22 : 23	+176 †	+132	—	—	—	5, 7

* Includes benzoates.

† The rotation of 7-dehydrocholesterol peroxide (Reference 18) is in good agreement with that for the dihydroergosterol peroxide and no doubt it is the specific rotation of ergosterol peroxide itself which is erroneous.

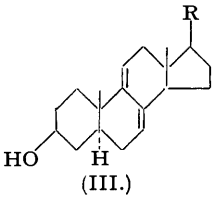
When a sterol contains two double bonds, one in the side chain and one in the nucleus, each makes a separate contribution to the Δ value observed on complete hydrogenation. Thus if the position of one double bond is known, it is a simple matter to deduce that of the second. Table IV illustrates this procedure. The Δ values for the nulcear ethenoid linkages in α-dihydroergosterol and α-spinasterol are in good agreement with the Δ^7 formulations (Barton, *loc. cit.*), but not with the values for Δ^8 or $\Delta^{8(14)}$ unsaturation, which double bond positions have formerly been proposed. Assuming the Δ^7 positions of the nuclear double bonds suggested in Part I for β- and δ-spinasterols, it is to be anticipated that a $C_{22:23}$ linkage is absent in these sterols, and that the side chain unsaturation is $C_{24:25}$ and $C_{25:26}$. It will be of interest to see whether these predictions will be verified, and whether the tentative suggestion of Ruigh (*Ann. Rev. Biochem.*, 1945, **14**, 225) that the spinasterols differ from each other in position of the nuclear unsaturation will be proved incorrect. The Δ values for the minor yeast sterols, assuming the positions of nuclear unsaturation detailed in Part I, are not incompatible with the side chain formulations suggested in the literature (Wieland, Rath, and Hesse, *Annalen*, 1941, **548**, 34). The optical rotatory power data for dihydroergosterols II and III are specially interesting. The Δ values for dihydroergosterol II agree with those for a $\Delta^{8(14)}$ double bond, but, having regard to the preparation of this sterol by the reduction of ergosterol, or the so-called ergosterol F, under alkaline conditions, such a formulation would be unexpected. Indeed, since both α-dihydroergosterol (dihydroergosterol I) and dihydroergosterol II give the same substance on epimerisation (Windaus *et al.*, *Annalen*, 1931, **488**, 91), and since dihydroergosterol II shows very weak absorption at 2400 A. (Windaus *et al.*, *Annalen*, 1930, **477**, 268) and has a somewhat lower m. p. than α-dihydroergosterol, it is strongly implied that the sterol II is only a slightly impure form of the α-sterol. Accordingly it is assumed below that dihydroergosterol II and α-dihydroergosterol are identical. Dihydroergosterol III was thought to possess a Δ^{14}-unsaturated linkage (Dithmar and Achtermann, *Z. physiol. Chem.*, 1932, **205**, 55; Heilbron, Johnstone, and Spring, *J.*, 1929, 2248), but the optical rotatory power data are in complete disagreement with this formulation (the Δ values for the nuclear unsaturation of the sterol and its acetate are +71 and +76 respectively, whereas values of −24 and −20 are to be anticipated according to Table I). The reason for these anomalies is discussed further below.

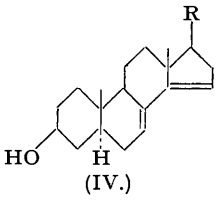
TABLE IV.

Substance.	⌐ positions.	Sterols. Δ values for both ⌐'s.	Sterols. ⌐ to be characterised.	Sterols. Δ value for other ⌐ (ex tables).	Sterols. Δ value for characterised ⌐.	Acetates. Δ values for both ⌐'s.	Acetates. ⌐ to be characterised.	Acetates. Δ value for other ⌐ (ex tables).	Acetates. Δ value for characterised ⌐.	Benzoates.* Δ values for both ⌐'s.	Benzoates.* ⌐ to be characterised.	Benzoates.* Δ value for other ⌐ (ex tables).	Benzoates.* Δ value for characterised ⌐.	Ref.
Brassicasterol	5 : 6, 22 : 23	+308	22 : 23	+251	*+57*	+314	22 : 23	+243	*+71*	+244	22 : 23	+173	*+71*	1
Poriferasterol	5 : 6, 22 : 23	+310	22 : 23	+251	*+59*	+319	22 : 23	+243	*+76*	+237	22 : 23	+173	*+64*	1
Stigmasterol	5 : 6, 22 : 23	+298	22 : 23	+251	*+47*	+310	22 : 23	+243	*+67*	+233	22 : 23	+173	*+60*	1
Zymosterol	9 : 11, 24 : 25	− 99	24 : 25	−100	*+ 1*	− 85	24 : 25	− 77	*− 8*	− 92	24 : 25	−103	*+11*	1
Fucosterol	5 : 6, 24 : 28	+269	24 : 28	+251	*+18*	+273	24 : 28	+243	*+30*	+187	24 : 28	+173	*+14*	1
α-Dihydroergosterol	7 : 8, 22 : 23	+144	7 : 8	+ 61	*+83*	+114	7 : 8	+ 61	*+53*	+122	7 : 8	+ 61	*+61*	1
α-Spinasterol	7 : 8, 22 : 23	+112	7 : 8	+ 61	*+51*	+ 92	7 : 8	+ 61	*+31*	+103	7 : 8	+ 61	*+42*	1
Episterol	7 : 8, S.C.†	+ 60	S.C.	+ 77	*−17*	+ 45	S.C.	+ 57	*−12*	+ 17	S.C.	+ 64	*−47*	1
β-Spinasterol	7 : 8, S.C.	+ 75	S.C.	+ 77	*− 2*	+ 46	S.C.	+ 57	*−11*	+ 63	S.C.	+ 64	*− 1*	1
δ-Spinasterol	7 : 8, S.C.	+ 75	S.C.	+ 77	*− 2*	+ 64	S.C.	+ 57	*+ 7*	+ 47	S.C.	+ 64	*−17*	1
Ascosterol	9 : 11, S.C.	−115	S.C.	−100	*−15*	− 70	S.C.	− 77	*+ 7*	−119	S.C.	−103	*−16*	1
Faecosterol	9 : 11, S.C.	−103	S.C.	−100	*− 3*	− 61	S.C.	− 77	*+16*	− 99	S.C.	−103	*+ 4*	1
Dihydroergosterol II	N.‡, 22 : 23	+ 96	N.	+ 61	*+35*	+ 79	N.	+ 61	*+18*	—	—	—	—	1, 3, 8
Dihydroergosterol III	N., 22 : 23	+132	N.	+ 61	*+71*	+137	N.	+ 61	*+76*	—	—	—	—	1, 9, 10

* Includes dinitrobenzoates. † Side chain. ‡ Nuclear bond.

Table V correlates the optical rotatory power evidence for a particularly interesting class of compounds. A large number of isomers of ergosterol have been described and, owing to the lability of the conjugated dienoid systems involved and the ambiguity of the methods of preparation, it has been difficult to assign structures to the majority of them. However physical evidence now enables a clear differentiation of most of these isomers to be effected, and there can be little doubt but that the formulæ suggested for these substances in this paper are substantially correct. For conjugated dienoid sterols containing the two double bonds in different rings (except between rings A and B) of the nuclear skeleton five distinct formulæ are possible [(III) to (VII) inclusive].


(III.)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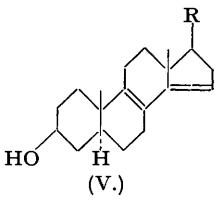
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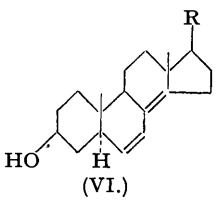
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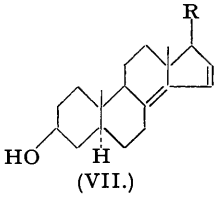
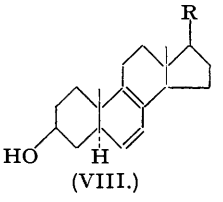
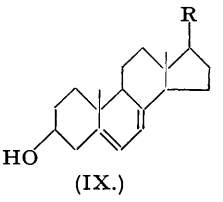
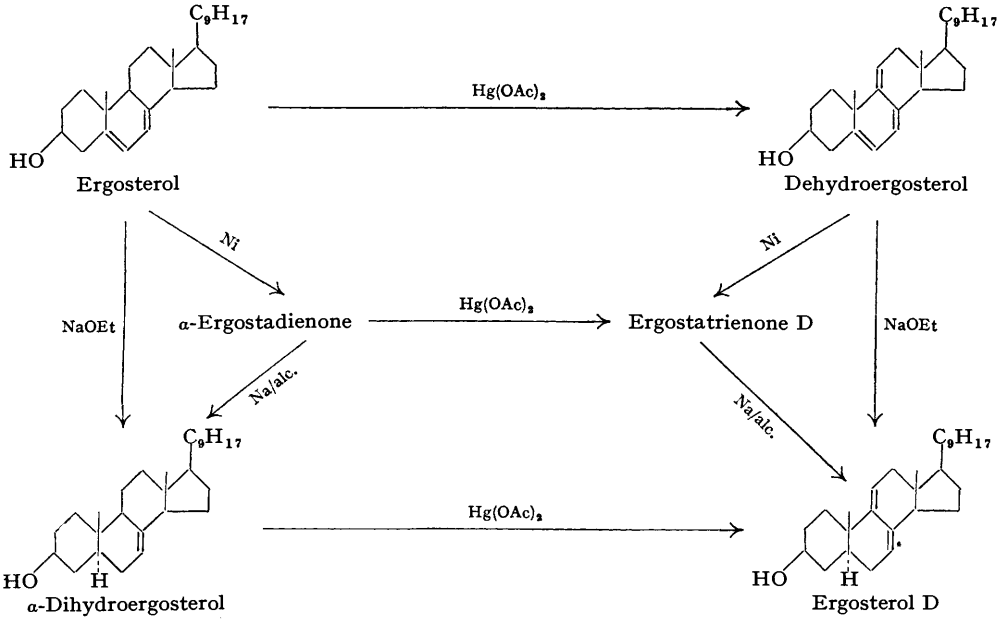

(VII.)


(VIII.)


(IX.)

The formula type (IV) for ergosterol B_3 and dehydrocholesterol B_3 is assigned because of the ease with which these sterols form maleic anhydride adducts, while all other compounds of the class under consideration [represented by (III), (V), (VI), (VII)] are without such affinity for philodienes. The formula (IV) for the B_3 sterols is supported by spectrographic evidence (see below), by the lead tetra-acetate experiments of Chen (*Ber.*, 1937, **70**, 1432), and by the parallel ease with which the comparable $\Delta^{8(14)}$-7 : 15-diketo system (Stavely and Bollenback, *J. Amer. Chem. Soc.*, 1943, **65**, 1285; compare the behaviour of amyradienedionol) forms a cyclic pyridazine derivative.

It has been the custom to formulate ergosterol D, one of the well-characterised ergosterol isomers, as (V; R = C_9H_{17}) (Callow, *J.*, 1936, 462) on the basis of the $\Delta^{8(14)}$ formula for its chemical progenitor, α-dihydroergosterol. Now that it has been conclusively shown that α-dihydroergosterol is not α-($\Delta^{8(14)}$) but γ-(Δ^{7}) olefinic, it is not surprising to find that optical rotatory power evidence definitely precludes the formula type (V) for ergosterol D.



It is possible to assign a definite formula to ergosterol D from purely chemical facts already recorded in the literature. Thus dehydroergosterol and ergosterol give ergosterol D and α-dihydroergosterol respectively on reduction with sodium ethoxide (Windaus *et al.*, *Annalen*, 1931, **488**, 91) while the corresponding ketones

are obtained by heating with metallic nickel (Windaus and Auhagen, *Annalen*, 1929, **472**, 185; Dithmar and Achtermann, *loc. cit.*). Ergosterol D derivatives are obtained also from the corresponding α-dihydroergosterol compounds by dehydrogenation with mercuric acetate (Heilbron, Johnstone, and Spring, *J.*, 1929, 2253; Windaus and Auhagen, *loc. cit.*; Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119). These interrelationships are more easily illustrated in the accompanying diagram and indicate strongly the correctness of (III) as the basic diene type for ergosterol D. The formulation receives additional support from the optical properties of the $\Delta^{7,9,22}$-ergostatrien-3(β) : 5-diol obtainable from dehydroergosterol (see Table V). The recently described cholestadiendiol (m. p. 228°) of Windaus *et al.* (*Annalen*, 1942, **552**, 135, 142) was formulated as a $\Delta^{7,9}$-diene. Such a formulation is quite contrary to the optical rotatory power evidence (Table V) and to the observed position of the ultra-violet absorption maximum at 2480 A. (see below).

Evidence in favour of the formula type (V) for dehydro-α-ergostenol is provided by general chemical reasoning (see below) and by the observed position of its ultra-violet absorption maximum. This maximum is at 2480 A. for both dehydro-α-ergostenol (Booker, Evans, and Gillam, *J.*, 1940, 1453) and for the analogous dehydro-α-cholestenol (Heath-Brown, Heilbron, and Jones, *J.*, 1940, 1482). Recently it has become widely accepted that increased substitution of a chromophore increases the wavelength of maximum absorption; within the range 2400—2500 A., which is characteristic of conjugated steroid dienes with two double bonds in different rings (Bergmann and Hirschmann, *J. Org. Chem.*, 1939, **4**, 40), this effect is readily apparent. Thus trisubstituted dienes, like $\Delta^{4,6}$-cholestadienol absorb at 2390 A. (Petrow, *J.*, 1940, 66; Spring and Swain, *ibid.*, 1941, 320), whilst the tetrasubstituted dienes ergosterol B_3 and ergosterol D have maxima at 2420 A. (Booker, Evans, and Gillam, *loc. cit.*; compare Woodward, *J. Amer. Chem. Soc.*, 1942, **64**, 72); the pentasubstituted dienes dehydro-α-ergostenol and dehydro-α-cholestenol, formulated as above, fall logically into the sequence. Ergosterol B_1 has likewise an absorption maximum at 2480 A.; its Δ values for nuclear unsaturation are remarkably close to those for dehydro-α-ergostenol (Table V). Accordingly it is suggested that ergosterol B_1 is (V; R = C_9H_{17}), and that dehydro-α-ergostenol is its 22 : 23-dihydro-derivative.

The other two types of diene represented by (VI) and (VII) are not well defined. The representation of cholestadienol C as (VI; R = C_8H_{17}) (Windaus, Linsert, and Eckhardt, *Annalen*, 1938, **534**, 22) is probably incorrect as it is likely that the labile material described under this heading was non-homogeneous. It may well be that ergosterol B_2 and the cholestadiendiol, m. p. 228°, mentioned above, should be represented by (VI), there being no examples of (VII) extant. However the formula type (V) for the cholestadienediol is not excluded. The formulation of ergosterol B_2 as (VI; R = C_9H_{17}) is also supported by its method of formation (see below). All the substances now grouped under (VI) have absorption maxima at 2480 A. which probably reflect the unique position of one of the double bonds in the chromophore as exocyclic to a five-membered ring (compare Woodward, *loc. cit.*).

The optical rotatory power data for ergosterols E, F, and G (Table V) as well as spectrographic and chemical evidence already recorded in the literature show that the formulæ tentatively suggested by Sobotka (" The Chemistry of the Sterids," p. 252 *et seq.*) for these substances must be rejected. Space restrictions do not justify the presentation of the detailed reasoning here, but it is clear that ergosterols E and F are non-homogeneous whilst ergosterol G, although its Δ values agree with those for $\Delta^{5,14}$-nuclear unsaturation, may well be similarly impure. Two other diethenoid sterols, tetrahydrodehydroergosterol and $\Delta^{5,14}$-ergostadienol, show anomalous Δ values (Table V) and there are additional good reasons for suggesting that these substances, as well, are non-homogeneous. α_1-Sitosterol, too, cannot have the suggested 5 : 6 and 14 : 15 double bonds (Bernstein and Wallis, *J. Amer. Chem. Soc.*, 1939, **61**, 2308) as the Δ values observed on reduction are very different from those to be anticipated (+260 for the sterol and +257 for the acetate). This supports the discrepancy already noted between the assigned structure of this substance and its optical rotation (compare Bernstein, Wilson, and Wallis, *J. Org. Chem.*, 1942, **7**, 103) and tends to confirm its nature as triterpenoid. A further confirmation of the relationship between neosterol and ergosterol (Part I) and an additional disproval of the suggested analogy with *iso*dehydrocholesterol (Wieland, Rath, and Hesse, *loc. cit.*) are provided by the data in Table V. Judging by the molecular rotation of neosterol this substance consists of a mixed crystal of ergosterol with about 20% of α-dihydroergosterol (compare Callow, *Biochem. J.*, 1931, **25**, 87).

Table VI lists steroid hydrocarbons containing one or two nuclear double bonds. For the first type of hydrocarbon the Δ values observed are not greatly different from those recorded for the corresponding sterols (see Table I). The Δ value for the reduction of the nuclear double bond in α-ergostadiene corresponds best to that for Δ^7-unsaturation, rather than to that for the previously suggested $\Delta^{8(14)}$-unsaturation. The fact that α-ergostadiene gives ergostatriene D (double bonds at 7 : 8 and 9 : 11) on mercuric acetate dehydrogenation supports this formulation. However, the molecular rotation difference recorded for the reduction of the nuclear double bond in β-ergostadiene (Heilbron, Spring, and Webster, *J.*, 1932, 1705) is far removed from that expected, as is the comparable constant for its chemical progenitor dihydroergosterol III (β-dihydroergosterol : see above). Both of these substances show Δ values which are very much greater than those predicted from their suggested formulæ (β-ergostadiene has Δ = + 142 for its nuclear unsaturation, whereas a value of about −25 is to be anticipated). Both can be obtained by the hydrogen chloride induced isomerisation of Δ^7-unsaturated steroids and both can be reduced to the corresponding $\Delta^{8(14)}$ compounds (compare the smooth and complete reduction of sterols which really do possess Δ^{14} unsaturation). Now while $\Delta^{8(14)}$-sterols are well known to rearrange smoothly to the Δ^{14}-isomers by the catalytic action of hydrogen chloride, there is good reason to believe that Δ^7-compounds should furnish a mixture of the corresponding Δ^5- and Δ^{14}-sterols.

TABLE V.

Substance.	Suggested formula: literature.	Suggested formula: this paper.	[M]D Sterol etc. Sterol.	[M]D Sterol etc. Acetate.	Δ_1.	[M]D Sterol etc. Stanol.	[M]D Sterol etc. Acetate.	Δ values. Sterol.	Δ values. Acetate.	References.
Ergosterol D	(V; R = C_9H_{17})	(III; R = C_9H_{17})	+ 83	+ 96	+ *13*	+ 64	+ 27	− *80* ¶	−*130* ¶	1, 8, 11, 12, 13, 14
epi-Ergosterol D *	(V; R = C_9H_{17})	(III; R = C_9H_{17})	+135	+197	+ *62*	+ 56	+ 93	−*140* ¶	−*165* ¶	8, 15, 16
$\Delta^{7,9,22}$-Ergostatrien-3(β) : 5-diol †	(III; R = C_9H_{17})	(III; R = C_9H_{17})	+198	+221	+ *23*	+ 77 ‡	+ 54 ‡	−*182* ¶	−*228* ¶	16, 17
Dehydrocholesterol B_3	(IV; R = C_9H_{17})	(IV; R = C_9H_{17})	−561	−490	+ *71*	+ 93	+ 60	+*654*	+*550*	1, 18
Ergosterol B_3	(IV; R = C_9H_{17})	(IV; R = C_9H_{17})	−792	−832	− *40*	+ 64	+ 27	+*795* ¶	+*798* ¶	1, 8, 13, 19
Dehydro-α-cholestenol	(V; R = C_8H_{17})	(V; R = C_8H_{17})	− 35	—	—	+ 93	—	+*128*	—	1, 20
Dehydro-α-ergostenol	(V; R = C_9H_{19})	(V; R = C_9H_{19})	− 67	−135	− *68*	+ 64	+ 27	+*131*	+*162*	1, 5, 11, 14, 21
Ergosterol B_1	—	(V; R = C_9H_{17})	−166	−241	− *75*	+ 64	+ 27	+*169* ¶	+207 ¶	1, 8, 9
Cholestadienol C	(? VI; R = C_8H_{17})	Impure	− 11	—	—	+ 93	—	+*104*	—	1, 22
$\Delta^{6,8(14)}$-Cholestadien-3(β) : 9-diol §	(VI; R = C_8H_{17})	(VI; R = C_8H_{17})	− 80	− 80	± 0	—	—	—	—	22
Ergosterol B_2	—	(VI; R = C_9H_{17})	−349	−350	− *1*	+ 64	+ 27	+*352* ¶	+*316* ¶	1, 8
$\Delta^{7,9}$-Cholestadien-3(β) : 6-diol ‖	(III; R = C_8H_{17})	(? VI; R = C_8H_{17})	−203	—	—	+140 ††	—	+*343*	—	23, 24, 25
Ergosterol E	(VII; R = C_9H_{17})	Impure	− 91 **	−166 **	− *75*	+ 64	+ 27	+ 94 ¶	+132 ¶	1, 26, 30
Ergosterol F	(VI; R = C_9H_{17})	Mixture	− 79	−105	− *26*	+ 64	+ 27	+ *82* ¶	+ *71* ¶	1, 16, 30
Ergosterol G	(VIII; R = C_9H_{17})	See text	−206	−276	− *70*	+ 64	+ 27	+*209* ¶	+*242* ¶	1, 27, 30
Tetrahydrodehydroergosterol	—	Mixture	− 52	− 40	+ *12*	+ 64	+ 27	+*116*	+ *67*	1, 28
$\Delta^{5,14}$-Ergostadienol	F's at 5 : 6 and 14 : 15	Impure	−115	−154	− *39*	+ 64	+ 27	+*179*	+*181*	1, 29
α_1-Sitosterol	F's at 5 : 6 and 14 : 15	Triterpenoid	− 8	+132	+*140*	+112	+179	+*120*	+ *47*	1
iso-Dehydrocholesterol	(VIII; R = C_8H_{17})	(VIII; R = C_8H_{17})	− 69	− 47	+ *22*	+ 93	+ 60	+*162*	+*107*	1
Neosterol	(VIII; R = C_9H_{17})	(IX; R = C_9H_{17})	−416	−294	+*122*	+ 64	+ 27	+*419* ¶	+*260* ¶	1

* The –OH at C_3 is, of course, in the (α) position in this substance.
† This substance has an additional –OH at the 5 position.
‡ These data are for 3 : 5-dihydroxycholestane and are unlikely to be far removed from those for the corresponding ergostane compound.
§ This substance has an additional –OH at the 9 position.
‖ This substance is said to possess an additional –OH at the 6 position.
¶ Corrected for side chain reduction (subtract 61).
** These rotations are for the 5461 A. line.
†† The value for cholestan-6-ol was used for this calculation: in general there is no difference between the molecular rotations of hydrocarbons and the corresponding sterols with a (β)-ol grouping at the 3 position.

TABLE VI.

Substance.	⊢'s to be characterised.	$[M]_D$. Unsaturated hydrocarbon.	$[M]_D$. Corresponding saturated hydrocarbon.	Δ value.	References.
Δ^2-Cholestene	2 : 3 *	+241	+89	−152	20, 31, 32, 33, 34, 35, 36, 37, 38
Δ^4-Cholestene	4 : 5	+248	+89	−159	20, 31, 32, 33, 34, 35, 39, 40
Δ^5-Cholestene	5 : 6	−207	+89	+296	20, 31, 32, 33, 34, 35, 41
Δ^6-*i*-Cholestadiene	6 : 7	−225	+93 †	+318	35, 42, 43
α-Ergostadiene	7 : 8	− 38	+77	+ 54 ‡	21, 44, 45, 46, 47
Δ^8-Cholestene	? 8 : 9	+ 41 §	+89	+ 48	20, 31, 32, 33, 34, 35, 48
α-Cholestene	8 : 14	+ 78 §	+89	+ 11	20, 31, 32, 33, 34, 35, 48
α-Ergostene	8 : 14	+ 42	+77	+ 35	21, 44, 45, 46, 47
β-Cholestene	14 : 15	+100 §	+89	− 11	20, 31, 32, 33, 34, 35, 48
β-Ergostene	14 : 15	+ 81	+77	− 4	21, 44, 45, 46, 49
$\Delta^{2,4}$-Cholestadiene	2 : 3, 4 : 5	+486	+89	−397	20, 31, 32, 33, 34, 35, 50
$\Delta^{3,5}$-Cholestadiene	3 : 4, 5 : 6	−460	+89	+549	20, 31, 32, 33, 34, 35, 37, 39, 51
$\Delta^{4,6}$-Cholestadiene	4 : 5, 6 : 7	+ 15	+89	+ 74	20, 31, 32, 33, 34, 35, 52
7-Dehydrocholestene	5 : 6, 7 : 8	−467	+89	+556	20, 31, 32, 33, 34, 35, 53
Ergostatriene D	7 : 8, 9 : 11	+163	+77	−147 ‡	21, 44, 45, 46, 47
$\Delta^{7,14}$-Cholestadiene	7 : 8, 14 : 15	−342 §	+89	+431	20, 31, 32, 33, 34, 35, 54
$\Delta^{8,14}$-Cholestadiene	8 : 9, 14 : 15	− 81 §	+89	+170	20, 31, 32, 33, 34, 35, 54
Dehydro-α-ergostene	8 : 9, 14 : 15	− 57	+77	+134	21, 44, 45, 46
Anomalous.					
β-Ergostadiene	?14 : 15	−126	+77	+142 ‡	21, 44, 45, 46, 47
$\Delta^{6,8(14)}$-Cholestadiene	? 6 : 7, 8 : 14	+ 4 §	+89	+ 85	20, 31, 32, 33, 34, 35, 54
$\Delta^{7,9}$-Cholestadiene	? 7 : 8, 9 : 11	+114 §	+89	− 25	20, 31, 32, 33, 34, 35, 54

* For evidence in favour of this double bond position see References 38 and 70.

† The value for *i*-cholesterol is used here; it is unlikely to be very far removed from that of the unknown *i*-cholestene.

‡ Corrected for side chain reduction.

§ These rotations in CCl_4; there is probably very little difference between these values and the true values in chloroform (see Eck and Thomas, References 55 and 56).

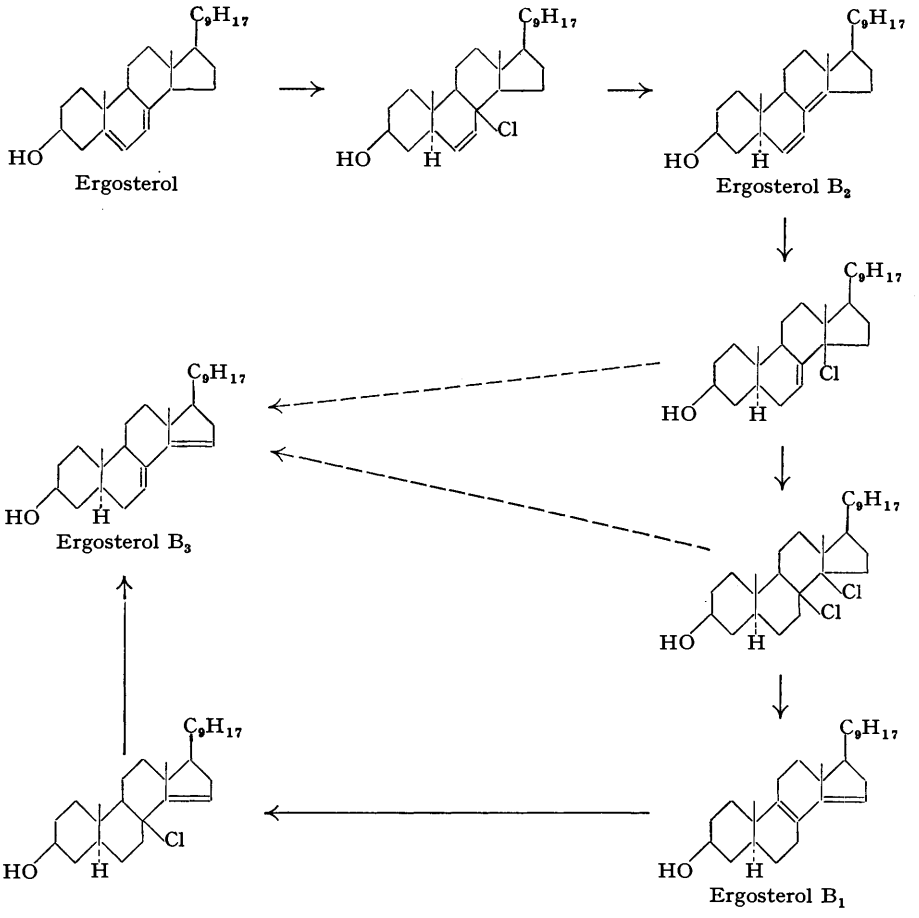
Thus zymostenol, which has one double bond in the ε- or Δ^9-position, furnishes a mixture of cholesterol and β-cholestenol on isomerisation with hydrogen chloride (Wieland and Benend, *Ber.*, 1942, **75**, 1708) and zymosterol itself behaves in an excatly similar manner (Wieland, Rath, and Benend, *Annalen*, 1942, **548**, 19). The presence of a substantial proportion of the Δ^5-steroid in dihydroergosterol III and β-ergostadiene, as well as a certain amount of the unrearranged material, would, of course, readily explain the unexpectedly high Δ values on reduction (compare Table I), and this interpretation of the behaviour of these compounds is suggested here as the reason for the optical anomalies observed.

Of the hydrocarbons containing two nuclear conjugated double bonds, the recently described cholestadienes of Eck and Hollingsworth (*J. Amer. Chem. Soc.*, 1942, **64**, 140) have values which, except in the case of $\Delta^{8,14}$-cholestadiene (dehydro-α-cholestene), are only roughly similar to those for the corresponding sterols. On the other hand the Δ values for 7-dehydrocholestene, dehydro-α-ergostene, and ergostatriene D are in good agreement with those for their respective sterol types (compare Table V). Of these cholestadienes the rotation for the $\Delta^{7,14}$ compound indicates that the material is, indeed, substantially of the assigned type, while the Δ values for the $\Delta^{6,8(14)}$- and $\Delta^{7,9}$-cholestadienes are, in proportion, far removed from those expected. Before discussing these further, it is desirable to give some explanation of the various methods of diene synthesis for steroids with the conjugation between two different rings (excepting between rings A and B) of the nucleus.

Three general classifications can be made. (A) Rearrangement of preformed diene systems. It is in this way that ergosterols B_1, B_2, and B_3 are formed (Windaus *et al.*, *Annalen*, 1931, **488**, 91) by the hydrogen chloride induced isomerisation of ergosterol itself. The reactions can be formulated as proceeding by 1 : 2 or 1 : 4 addition and 1 : 2 elimination as in the diagram. In this way the formation of ergosterol B_3 from ergosterols B_1 and B_2 is readily explained (Windaus *et al.*, *loc. cit.*). (B) Direct dehydrogenation by mercuric acetate, apparently without double bond migration. Examples have already been given above. (C) Reactions proceeding by hydroxylation of a double bond followed by two-stage elimination, or elimination, allylic rearrangement, and further elimination. Examples are shown in the diagram for the hydroxylated compounds, but exactly similar mechanisms can be postulated with bromine, etc.

Both the $\Delta^{7,9}$- and the $\Delta^{6,8(14)}$-cholestadienes mentioned above were synthesised by methods that cannot be reconciled with any of these classifications. Thus the $\Delta^{7,9}$-diene is said to be prepared by the action of mercuric acetate on $\Delta^{8(9)}$-cholestene (the preparation using bromine cannot be criticised on these grounds). However, if the $\Delta^{8(9)}$-cholestene is substantially Δ^7-cholestene as previously suggested (Part I) the discrepancy is clearly resolved. Probably, in actual fact, the so-called $\Delta^{8(9)}$-cholestene is a mixture in which the Δ^7-isomer is predominant. Eck and Hollingsworth (*loc. cit.*) describe the preparation of the $\Delta^{6,8(14)}$-cholestadiene by the action of alcoholic hydrochloric acid on $\Delta^{8(9)}$-cholesten-7-ol, a reaction which should, according to the above classification, furnish the already mentioned $\Delta^{7,9}$-isomer. However, the identity of $\Delta^{8(9)}$-cholesten-7-ol

itself is by no means proved, as its structure depends on the correctness of the formulation of $\Delta^{8(9)}$-cholesten-7-one. This ketone is said to possess a specific rotation of +4° (Eck and Hollingsworth, *J. Amer. Chem. Soc.*,



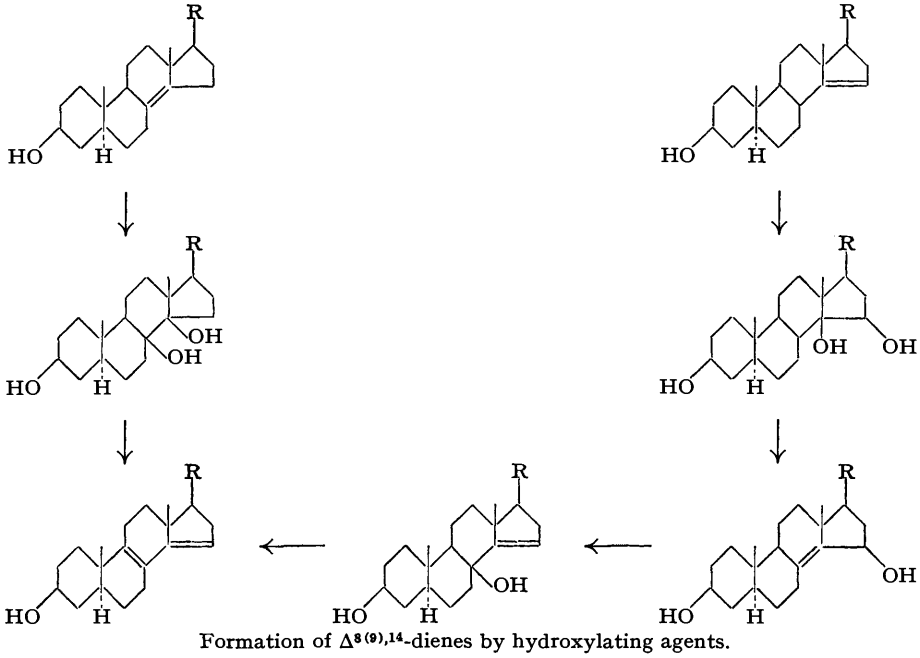
1941, **63**, 2986), so that the molecular rotation difference with respect to cholestane is +74. The exactly analogous $\Delta^{8(9)}$-7-keto compounds of Stavely and Bollenback (*ibid.*, 1943, **65**, 1290, 1600) have, however, marked specific lævorotations (of the order −40°) and Δ values of +192 ± 15. It would be unwise therefore to consider the $\Delta^{7,9}$- and $\Delta^{6,8(14)}$-cholestadienes to be chemical individuals until further evidence as to their purity is available.

TABLE VII.

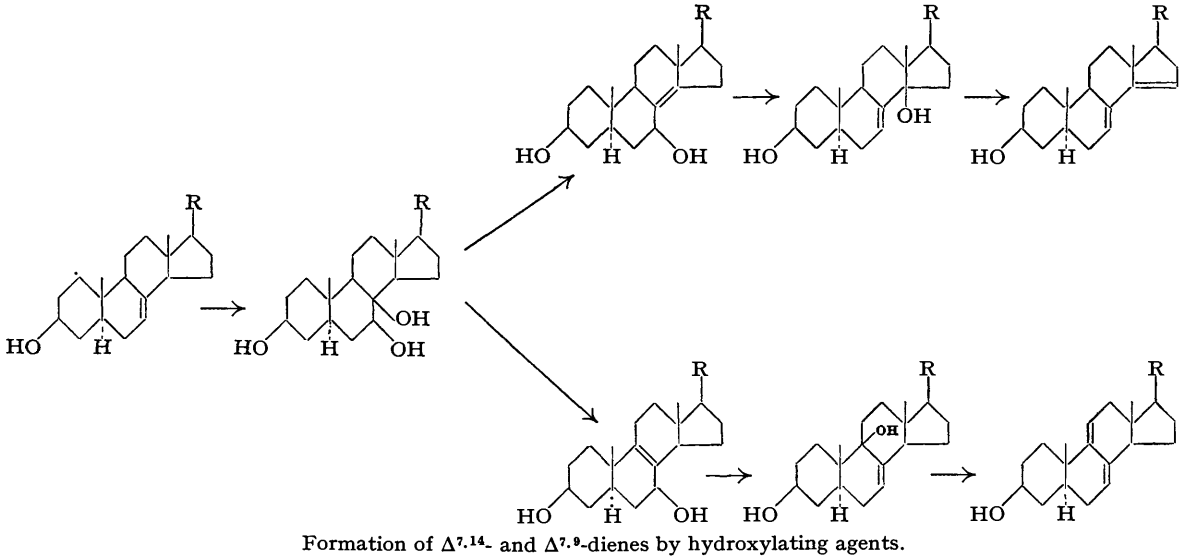
Diene type.	Position of ⊨'s.	Δ values of ⊨'s. First.	Second.	Sum.	A.M. of diene Δ values (ex tables)	No. of examples.	Limits of variation from A.M.	References.
$\Delta^{2,4}$-Stadienes	2 : 3, 4 : 5	−152	−159	−311	−397	1	—	20, 31, 32, 33, 34, 35
$\Delta^{3,5}$-Stadienes	3 : 4, 5 : 6	−101 *	+251	+150	+549	1	—	20, 31, 32, 33, 34, 35, 69
$\Delta^{4,6}$-Stadienes	4 : 5, 6 : 7	−159	+200 *	+ 41	+ 74	1	—	20, 31, 32, 33, 34, 35
$\Delta^{5,7}$-Stadien-3(β)-ols ...	5 : 6, 7 : 8	+251	+ 77	+328	+538	5	+40 to −40	1
$\Delta^{6,8}$-Stadien-3(β)-ols ...	6 : 7, 8 : 9	+200 *	+ 48	+248	+162	1	—	1
$\Delta^{6,8(14)}$-Stadien-3(β)-ols	6 : 7, 8 : 14	+200 *	+ 9	+209	+352	1	—	1
$\Delta^{7,9}$-Stadien-3(β)-ols ...	7 : 8, 9 : 11	+ 77	−100	− 23	−134	3	+54 to −48	1
$\Delta^{7,14}$-Stadien-3(β)-ols ...	7 : 8, 14 : 15	+ 77	− 24	+ 53	+725	2	+70 to −71	1
$\Delta^{8,14}$-Stadien-3(β)-ols ...	8 : 9, 14 : 15	+ 48	− 24	+ 24	+143	3	+26 to −15	1

* These values have been assigned. That for the Δ^{3}-bond is calculated from the molecular rotations recorded for Δ^{3}-cholesten-3 : 4-diol (Reference 34) and the *trans*-cholestan-3 : 4-diol of Rosenheim and Starling (Reference 69). The value for the Δ^{6}-bond is certainly of the right sign and probably of the right magnitude. Compare the rotations of Δ^{6}-*i*-cholestadiene and Δ^{6}-cholesten-3(β) : 5-diol (References 57 and 58).

The correlation of the optical rotatory power data on sterol dienes has led to the development of an interesting rule, which enables a clear prediction to be made as to whether the rotation of the diene will be less or greater than that of the corresponding saturated analogue. The operation of this generalisation, which may be called the " dichromophore rule," is illustated in Table VII. This rule states that, for any sterol containing two unit chromophores in conjugation, the algebraic sum of the Δ values for the two individual



Formation of $\Delta^{8(9),14}$-dienes by hydroxylating agents.



Formation of $\Delta^{7,14}$- and $\Delta^{7,9}$-dienes by hydroxylating agents.

chromophores is always of the same sign and often of the same magnitude as the Δ value for the transformation of the conjugated dichromophore to its saturated analogue. This generalisation is equally applicable to αβ-unsaturated ketones (Table VIII), although there is one exception which, it is suggested, is due to serious impurity. In all cases save two the Δ values for the conjugated dichromophore is greater than the sum of the Δ values for the individual chromophores and this fact may prove to be a further extension of the scope of the rule. The generalisation cannot be extended to three chromophores in straight conjugation, but is valid for all the examples so far listed in the sterol field of three chromophores in cross-conjugation.

The possible operation of " vicinal action " in affecting the optical rotatory powers of the compounds mentioned in this paper will be discussed in a further communication.

TABLE VIII.

Unsaturated ketone type.	Δ values for individual chromophores.	F.	Δ values for individual chromophores. Ketone.	Sum.	A.M. of dichromophore Δ values.	No. of examples.	Limits of variation from A.M.	References.
Δ^1-3-Ketones		—	− 69	—	−161	1	—	1, 59, 60
Δ^4-3-Ketones		−159	− 69	−228	−259	2	+9 to −9	1
Δ^4-6-Ketones *		− 78 †	+153	+ 75	+145	1	—	1, 57, 61, 68
Δ^5-7-Ketones ‡		+243	+220	+463	+489	1	—	1, 62
Δ^8-7-Ketones ‡		± 0	+220	+220	+192	2	+15 to −15	1, 63, 64
$\Delta^{8(14)}$-7-Ketones ‡		+ 9	+220	+229	+318	3	+16 to −16	1, 63, 64, 65, 66
$\Delta^{8(14)}$-15-Ketones ‡		+ 9	—	—	−462	2	+1 to ±0	1, 66, 67
Anomalous.								
3 : 7-Diketo-$\Delta^{8(14)}$-ergosten-5-ol		+ 24	+220	+244	−193 §	—	—	1, 29

* Values for the 3(β)-ol derivatives.
† This value is calculated using the molecular rotation of *allo*-cholesterol (Reference 68) in C_6H_6.
‡ Values for the 3(β) acetates.
§ Calculated using the recorded molecular rotation of 3 : 5-dihydroxycholestane (Reference 17).

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