248. *The Application of the Method of Molecular Rotation Differences to Steroids. Part III. Steroidal Hormones and Bile Acids.*

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The data available in the literature on the optical rotatory powers of steroidal hormones and bile acids have been correlated, and comparison is made with similar work in the sterol field (see Barton, Part I, *J.*, 1945, 813; Part II, this vol., p. 512). A comparatively small number of the steroids considered in this paper possess anomalous rotatory powers which, it is suggested, are due to the preparations concerned being impure.

Physical considerations lead to the formulation of *u*-ergostane and its derivatives and of coprostadienols B and D. The application of optical rotatory power evidence in the unsaturated bile acid field results in a clarification of conflicting data and the formulation of various substances including the α- and β-dihydroxycholadienic acids and β-apocholic acid.

Finally there is a short note on the relationship between melting point and structure in the steroid field and, as an example, the characterisation of the nuclear double bond of the mixed stellasterols as Δ^7 is mentioned.

Previous communications (Barton, *locc. cit.*) have shown how useful a critical study of optical rotatory power can be in the steroid field. All the substances so far considered have, however, been members of the *allo*-cholane type with rings A and B fused in the *trans*-position and with the 3 hydroxyl in the (β) configuration [see formula (I) below]. Many other naturally occurring substances of the steroid group have different arrangements in these two rings. For example androsterone is of type (II), the oestrogenic hormones have ring A, aromatic, the bile acids are of type (IV), whilst, for contrast, progesterone, corticosterone, and testosterone are Δ^4-3-ketones. Representatives of all the four types [formulæ (I) to (IV) below] are found in the cardiac aglycone and steroidal sapogenin fields. Δ^5-Steroids of type (V) occur in Nature and are possible precursors of many of the other steroids, but compounds of type (VI) are rarely met even in synthetic work.

Just as the optical rotation changes on acylation of the C_3 hydroxyl are characteristic in differentiating naturally occurring sterols from triterpenes (Part I), so are the corresponding rotational changes for substances of types (II) to (IV) uniquely characteristic of the molecular environment of the hydroxyl in ring A. Owing, however, to the limited recording of rotational data only Δ_1 values (for definition see Part I) can be considered in detail; these are summarised in Table I. All the relevant observations in the literature have been collected for bile acids and steroidal hormones and treated as described previously (Part I), although, in order to conserve space, compounds are not mentioned individually. The term "bile acid" is used generically to include esters and other closely related derivatives that have been prepared in recent years, whilst the term "steroidal hormone" includes the natural androgenic hormones and all natural and synthetic analogues in both the androstane and the pregnane series. Rotational values in chloroform have not usually been available and comparison has been made, therefore, between the values recorded for the acetate and its parent alcohol both in some other solvent.

TABLE I.

Substance class.	Formula type.	Solvent.	A.M.* of Δ_1 values.	No. of examples.	Limits of variation from A.M.	References.
Stanols	(I)	$CHCl_3$	−34	7	+ 8 to −11	1.
Bile acids and steroidal hormones †	(I)	All	−17	12	+16 to −20	2, 3, 4, 5, 6, 7, 8, 9, 10, 11.
Stanols	(II)	$CHCl_3$	+29	2	+ 8 to − 9	12, 13, 14.
Bile acids and steroidal hormones †	(II)	All	+24	7	+13 to −23	4, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25.
Bile acids †	(III)	Me_2CO	+17	5	+13 to −17	26, 27, 28, 29, 30.
Stanols	(IV)	All	+77	3	+14 to −18	31, 32.
Bile acids and steroidal hormones †	(IV)	All	+83	21	+23 to −29	26, 27, 29, 33, 34, 35, 36, 37, 38, 39, 40, 41.
Δ^5-Stenols	(V)	$CHCl_3$	−35	12	+15 to −10	1.
Bile acids and steroidal hormones †	(V)	All	−28	32	+25 to −23	2, 3, 6, 8, 33, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63 64, 65, 66, 70.

* Arithmetic mean. All rotations are for the Na_D line.
† For definitions of compounds included under these headings see text.

There is good reason, however, to believe, that Δ_1 values in chloroform are not very different from those in other solvents (cf. Plattner and Heusser, *Helv. Chim. Acta*, 1944, **27**, 748) and a number of determinations have

TABLE II.

Substance.	Δ_1 values. Acetone.	Alcohol.	Chloroform.	Dioxan.
Cholesterol	−35	−21	−34 (−35 *)	−32
Dehydro*iso*androsterone	—	−17	−29	—
Cholestanol	−31	−31	−29 (−34 *)	−28
*iso*Androsterone	—	−27	−32	—

* Mean values from analysis of literature (see Part I).

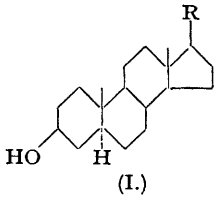
been made (Table II) which confirm this view. Most of the examples quoted in Table I are substituted in the side chain and/or in ring C, but no striking and unambiguous examples of vicinal action can be reported.

TABLE III.

Substance.	Formula type.	Solvent.	$[M]_D$. Alcohol.	Acetate.	Δ_1.	Refs.
Bile acids :						
3(β) : 11(β) : 17(β)-Trihydroxy*allo*ætiocholanic acid methyl ester	(I)	$C_4H_8O_2$	+ 29	+ 45	+ 16	4
3(α)-Hydroxybisnor*allo*cholanic acid methyl ester	(II)	$CHCl_3$	+ 43	+ 40	− 3	12
3(β)-Hydroxy-11-ketocholanic acid methyl ester	(III)	Me_2CO	+158	+250	+ 92	67
3(α)-Hydroxy-12-ketonorcholanic acid	(IV)	$C_4H_8O_2$	+263	+418	+155	68
3(α)-Hydroxy-12-ketobisnorcholanic acid	(IV)	$C_4H_8O_2$	+308	+267	− 41	68
3(β) : 17-Dihydroxy-Δ^5-androsten-17-acetic acid methyl ester	(V)	$CHCl_3$	−322	−275	+ 47	47
3(β)-Hydroxy-$\Delta^{5,16}$-ætiocholadienic acid methyl ester	(V)	$CHCl_3$	−165	−257	− 92	42
Steroidal hormones :						
3(β)-Hydroxy-20-*iso*ternor*allo*cholanyl methyl ketone	(I)	$CHCl_3$	+ 41	+ 46	+ 5	6
*allo*Pregnan-3(α)-ol-20-one	(II)	C_2H_5OH	+280	+342	+ 62	10
Pregnan-3(β)-ol-20-one	(III)	C_2H_5OH	+321	+310	− 11	39
Δ^5-Pregnen-3(β)-ol-20-one-21-al diethyl mercaptal	(V)	Me_2CO	+602	+717	+115	49
21-Bromacetoxy-Δ^5-pregnen-3(β)ol-20-one	(V)	$CHCl_3$	+113	+129	+ 16	69
3(β)-Hydroxy-Δ^5-20-*iso*ternorcholenyl phenyl ketone	(V)	$CHCl_3$	−191	−193	− 2	48
3(β)-Hydroxy-Δ^5-ternorcholenyl mesityl ketone	(V)	$CHCl_3$	−278	−264	+ 14	48
3(β)-Hydroxy-Δ^5-ternorcholenyl methyl ketone	(V)	$CHCl_3$	−240	−307	− 67	48
3(β)-Hydroxy-Δ^5-ternorcholenyl *iso*amyl ketone	(V)	$CHCl_3$	−219	−277	− 58	48

A number of bile acid and steroidal hormone derivatives have Δ_1 values which are completely incompatible with their allotted structures. These are listed in Table III, and it is confidently predicted that repetition of the preparation of these substances will lead to extensive revision of their recorded rotations.

The predicted revised Δ_1 values can, of course, be read off from Table I. It is significant that 5 of the 16 cases of anomalous Δ_1 values are drawn from one investigation (references 6 and 48).



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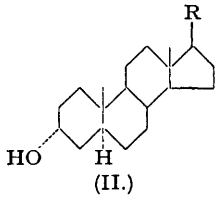

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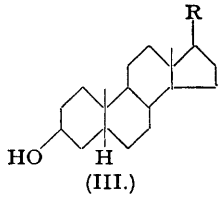

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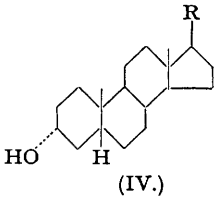

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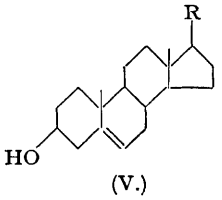

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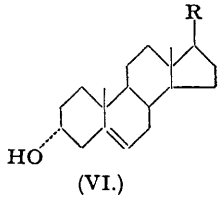


(VI.)

Although, as mentioned above, Δ_1 values are roughly the same in all the solvents examined, it is not necessarily true that Δ values in general are the same in all solvents. Thus there is a small, but definite, decrease in the Δ values for the reduction of Δ^5 double bonds when rotations in chloroform are compared with those in other solvents (Table IV). This effect can also be seen from analysis of the recorded literature values

TABLE IV.

Substance.	Δ values for reduction of Δ^5 double bond. Acetone.	Alcohol.	Chloroform.	Dioxan.
Cholesterol	+229	+229	+243 (+251 *)	+228
Dehydro*iso*androsterone	—	+245	+255	—
Cholesteryl acetate	+223	+219	+248 (+243 *)	+232
Dehydro*iso*androsterone acetate	—	+229	+252	—

* Mean values from analysis of literature (see Part II).

in the bile acid and steroidal hormone fields, but space restrictions do not justify such detailed considerations here. In general the Δ values for this reduction in the steroidal hormone and bile acid fields are those which can be predicted from Table IV. Anomalous cases, for this transformation, are comparatively few and most are included in references 6 and 48 (cf. above). In view of the wide recording of sterol rotations in chloroform solution it is generally desirable that this solvent should be used for the other types of steroid as well in order that comparison between compounds may be facilitated.

It is possible to compile lists of Δ values for numerous other transformations in the bile acid and steroidal hormone series, but such catalogues serve only to illustrate already enunciated principles and the chemical interest of the subject matter does not justify the mere presentation of lists of substances with anomalous rotatory power. The verification of the incorrectness of the rotations of the substances detailed in Table III will suffice to confirm the validity of the molecular rotation difference method in this field, and other anomalous cases can be corrected by individual workers as the preparation of such compounds is repeated. In any case it is a simple matter to make correlations on the particular molecular change in which one is interested. One generalisation is, however, worth mentioning, *viz.*, that with few exceptions there is practically no difference between the molecular rotations of bile acids and their esters. This is in agreement with the general position in the triterpenoid field (cf. Barton and Jones, *J.*, 1944, 659).

The problem of the formulation of the various derivatives of *u*-ergostanol first described by Windaus and Auhagen (*Annalen*, 1929, **472**, 185) is easily solved by the use of molecular rotation data. That *u*-ergostanol itself is *epi*coproergostanol was suggested by Laucht (*Z. physiol. Chem.*, 1937, **246**, 171) although unambiguous proof was not given. The molecular rotation and melting point of *u*-ergostanol (Windaus and Auhagen, *loc. cit.*) are both too high for the interpolated data for *epi*coproergostanol deduced by the Methods of Differences (for melting point considerations see below), whilst for the *epi*coproergostanol of Wetter and Dimroth (*Ber.*, 1937, **70**, 1665) the physical constants recorded are those expected. Saturated derivatives of cholestanol and ergostanol differ from each other by about 20 to 30 in molecular rotation (the cholestanol compounds being the more positive; see Part I, Table I). *u*-Ergostanol acetate and *u*-ergostane are lower in molecular rotation by 28° and 23° respectively than their corresponding *epi*coprosterol homologues. The agreement here and in the difference (23°) between the molecular rotation of Wetter and Dimroth's *epi*coproergostanol and that of *epi*coprosterol is a strong indication that *u*-ergostane is coproergostane and that *u*-ergostanol acetate is *epi*coproergostanol acetate. The original *u*-ergostanol must have been impure and the description (the acetylation of *u*-ergostanol has never been described) of its preparation is in keeping with this view. Furthermore *u*-ergostanol acetate and Wetter and Dimroth's *epi*coproergostanol acetate have the same melting point.

Accepting these identities the formulæ (VII; R = C_9H_{17}) and (VIII; R = C_9H_{17}, R′ = H) must be assigned to *u*-ergostadienol and to *u*-ergostatrienol respectively. Similarly *u*-ergostatrienol B must be given the formula (IX; R = C_9H_{17}, R′ = H) for it absorbs at 2480 A. (Dithmar and Achtermann, *Z. physiol. Chem.*, 1932, **205**, 55) and does not react with maleic anhydride (compare *u*-ergostatrienol which absorbs at 2420 A., and see Part II). The methods of preparation of all these substances are in agreement with these formulations (cf. Windaus, *Annalen*, 1927, **453**, 101, and see Part II). The Δ values for all these compounds are set out in Table V. The

TABLE V.

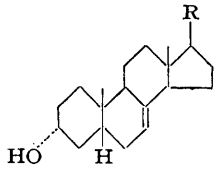
Substance.	Suggested formula; this paper.	$[M]_D$ in $CHCl_3$. Alcohol.	$[M]_D$ in $CHCl_3$. Acetate.	Δ_1.	$[M]_D$ in $CHCl_3$. Reduced alcohol.	$[M]_D$ in $CHCl_3$. Reduced acetate.	Δ values. Alcohols.	Δ values. Acetates.	Refs.
u-Ergostanol	(IV; R = C_9H_{19})	+137	+178	+ *41*	—	—	—	—	75, 76, 77
		+101*	+178	+ *77*	—	—	—	—	
*epi*Coprosterol ...	(IV; R = C_8H_{17})	+124	+206	+ *82*	—	—	—	—	31
u-Ergostadienol ...	(VII; R = C_9H_{17})	+187	+255	+ *68*	+101*	+178	−147†	−138†	75, 76, 77, 78
u-Ergostatrienol ...	(VIII; R = C_9H_{17}, R′ = H)	+349	+451	+*102*	+101	+178	−309†	−334†	75, 76, 77
Coprostadienol D ‡	(VIII; R = C_8H_{17}, R′ = H)	+361	—	—	+100	—	−261	—	79, 80, 81
u-Ergostatrienol B	(IX; R = C_9H_{17}, R′ = H)	−194	−158	+ *36*	+101	+178	+234†	+275†	75, 76, 77
Coprostadienol B ‡	(IX; R = C_8H_{17}, R′ = H)	−184	−209	− *25*	+100	—	+284	—	79, 80, 81

* As discussed in the text this value, recorded for *epi*coproergostanol, should be identical with that for pure *u*-ergostanol.

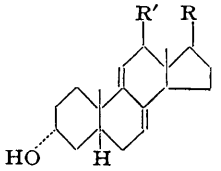
† Corrected for side chain reduction (see Reference 93).

‡ These substances have, of course, a 3(β)-ol grouping.

change in Δ value from about +60 in the *allo*cholane series (Part II) to −140 in the cholane series for the reduction of the Δ^7 double bond is particularly remarkable.



(VII.)



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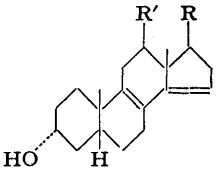

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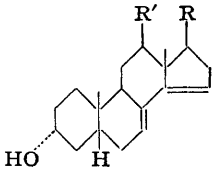

(X.)

Recently Windaus and Zühlsdorff (*Annalen*, 1938, **536**, 204) have described the thorough characterisation of $\Delta^{6:8(9)}$-coprostadien-3(β)-ol and its rearrangement to coprostadienol B. On the grounds of optical rotation (Table V) this latter substance must now be assigned the formula [IX; R = C_8H_{17}, R′ = H and 3(β)-ol] in agreement with its absorption maximum at 2480 A. and its failure to react smoothly with maleic anhydride. A by-product of the treatment of $\Delta^{6:8(9)}$-coprostadienol-3(β)-ol with maleic anhydride was a diene alcohol, m. p. 128—129°, absorbing at 2400 A. The optical rotation (Table V) clearly shows, in agreement with the absorption maximum, that this substance should be called coprostadienol D and be formulated as [VIII; R = C_8H_{17}, R′ = H and 3(β)-ol].

The literature contains descriptions of a large number of mono-unsaturated bile acid derivatives. All those that can be treated by the Method of Molecular Rotation Differences are recorded in Table VI and it is possible by this procedure to effect a reduction in the number of substances worthy of consideration as homogeneous entities. In the *allo*cholane series the only double bond position in the molecule which resists catalytic reduction in acetic acid solution at room temperature is at $\Delta^{8(14)}$. Double bonds in other parts of the molecule are either reduced under these conditions or isomerised to the $\Delta^{8(14)}$ compounds. In the cholane series the situation is somewhat different in that Δ^7 compounds seem to be rearranged only sluggishly to the $\Delta^{8(14)}$ isomers by this procedure. Thus among the cholenic acids three are recorded (β-, γ-, and δ-cholenic acids) which resist hydrogenation with either platinum or palladium catalysts, whilst two similar lithocholic and three deoxycholic acid derivatives are reported (see Table VI). β-, γ-, and δ-Cholenic acids have Δ values for the reduction of the double bond of from −56° to −75°, and are best regarded as mixtures of Δ^7 and $\Delta^{8(14)}$ acids. The other possible position of the double bond at $\Delta^{8(9)}$ is most unlikely, for Windaus and Zühlsdorff (*loc. cit.*) have shown that δ-coprostenol (for Δ value see Table VI) is smoothly rearranged by hydrogenating catalysts, as is δ-cholestenol, to give the α-isomer (double bond shift from $\Delta^{8(9)}$ to $\Delta^{8(14)}$). The Δ value for the reduction of the double bond in α-coprostenol cannot be obtained by direct comparison, but by deduction from the recorded values for the molecular rotations of the acetate and dinitrobenzoate it is clear that practically no change in rotation is caused by this transformation. The cholenic acids under consideration, therefore, are mixtures of $\frac{1}{3}$ to $\frac{1}{2}$ of the Δ^7 isomer with $\frac{2}{3}$ to $\frac{1}{2}$ of the $\Delta^{8(14)}$ isomer. Although the *apo*chenodeoxycholic acids (Takahashi, *Z. physiol. Chem.*, 1938, **255**, 277; Yamasaki and Takahashi, *ibid.*, 1938, **256**, 21) differ in melting point it seems that both are mainly the Δ^7 isomer. The formulation of *apo*cholic and β-*apo*cholic acids is specially interesting. For chemical reasons summarised by Callow (*J.*, 1936, 462) *apo*cholic acid must be

TABLE VI.

Substance.	⊨ position : literature.	⊨ position : this paper.	Solvent.	$[M]_D$. Unsaturated acid.	$[M]_D$. Saturated acid.	Δ (in cholane series).	Δ (in *allo*-cholane series).	References.
Δ^2-Cholenic acid	2 : 3	2 : 3	C_2H_5OH	+ 61	+102†	+ 41	−152	83, 84, 85
Δ^3-Cholenic acid	3 : 4	3 : 4	C_2H_5OH	+ 68	+102	+ 34	—	83, 84, 85
Δ^5-Cholenic acid	5 : 6	5 : 6	C_2H_5OH	−240	+102	+342	+296	83, 84, 85
β-Cholenic acid ‡	8 : 14	Mixed 7 : 8 and 8 : 14	$CHCl_3$	+140	+ 79	− 61	—	84, 85, 86
γ-Cholenic acid ‡	—	Mixed 7 : 8 and 8 : 14	$CHCl_3$	+154	+ 79	− 75	—	84, 85, 87
δ-Cholenic acid ‡	8 : 14§	Mixed 7 : 8 and 8 : 14	C_2H_5OH	+158	+102	− 56	—	84, 85, 88
*apo*Chenodeoxycholic acid ‡	8 : 14	Mainly 7 : 8	C_2H_5OH	+266	+128	−138	+ 77 ‖	32, 72, 73, 86
β-*apo*Chenodeoxycholic acid ‡	8 : 14§	Mainly 7 : 8	C_2H_5OH	+288	+128	−160	+ 77 ‖	32, 72, 73, 88
*apo*Cholic acid ‡	8 : 9 or 8 : 14	8 : 14	C_2H_5OH	+183	+220	+ 37	+ 9 ‖	89, 90, 91, 92, 93, 94, 95
β-*apo*Cholic acid ‡	8 : 14§	7 : 8	C_2H_5OH	+308	+220	− 88	+ 77 ‖	89, 96
Dihydroxycholenic acid	7 : 8 or 14 : 15	14 : 15	C_2H_5OH	+238	+220	− 18	− 24 ‖	89, 91, 94, 95, 96, 97, 98, 99
*iso*Dihydroxycholenic acid ‡	8 : 9	Rearrangement	C_2H_5OH	+ 23	+220	+197	—	89, 95, 98, 99, 100
3(α)-Bile acids (cholane * series)	9 : 11	9 : 11	All	—	—	− 48	−100 ‖	32, 36, 71, 72, 73, 74
3(α)-Bile acids (cholane * series)	11 : 12	11 : 12	All	—	—	− 26	—	26, 27, 30, 32, 36, 71, 72, 73
3 : 7 : 22-Trihydroxy-Δ^{14}-cholene	14 : 15	14 : 15	C_2H_5OH	+128	+121	− 7	− 24 ‖	101
δ-Coprostenol	8 : 9	8 : 9	$CHCl_3$	+ 58	+100	+ 42	+ 48 ‖	79, 80, 81

* Three examples, in good agreement with each other, are available in both series (see References cited).
† This rotation is assigned from a knowledge of the rotation in $CHCl_3$ (compare Reference 33).
‡ These substances resist catalytic hydrogenation.
§ With C_9 epimerism.
‖ These values apply to the corresponding 3(β)-ol compounds, but will not be very different from the true Δ values for the analogous 3(α)-ol substances.

either $\Delta^{8(9)}$ or $\Delta^{8(14)}$. The failure of *apo*cholic acid to rearrange under the influence of hydrogenating catalysts in acetic acid solution is a clear confirmation of the views of Callow (*loc. cit.*) that it has a $\Delta^{8(14)}$ bond. Optical rotatory power evidence (cf. Callow and Young, *Proc. Roy. Soc.*, 1936, *A*, **157**, 194) is not in disagreement with this formulation, but clearly shows in addition that β-*apo*cholic acid is substantially the Δ^7 isomer (compare Tables V and VI) and not the C_9 epimer of normal *apo*cholic acid as suggested by Callow (*loc. cit.*). It is clear also that the formulation of dihydroxycholenic acid as the Δ^{14} analogue of *apo*cholic acid is correct. Thus chemical evidence shows that dihydroxycholenic acid must be either Δ^7 or Δ^{14} unsaturated, but optical evidence (Table VI) as well as the facile catalytic reduction of this substance prove that the Δ^7 bond cannot be present in spite of the persistent formulations of Japanese workers to this effect (see, *e.g.*, papers in *J. Biochem. Japan*, 1939, etc.). *iso*Dihydroxycholenic acid is unique amongst the isomers of *apo*cholic acid in having a large positive Δ value on reduction (assuming a relationship to deoxycholic acid) and it is impossible to reconcile this with any double bond position in the cholane skeleton (Table VI). Previously the most logical formulation for this acid was as the $\Delta^{8(9)}$ isomer, but a comparison with δ-coprostenol precludes this position. *iso*Dihydroxycholenic acid, moreover, is not rearranged to *apo*cholic acid under hydrogenating conditions, or by hydrogen chloride or light. Its reaction with bromine is likewise peculiar. It is to be concluded that *iso*dihydroxycholenic acid is formed by some rearrangement of the *apo*cholic acid molecule and that its catalytic perhydrogenation will not be found to yield deoxycholic acid.

The conjugated dihydroxycholadienic acids, derived by the action of oxidising agents on α-*apo*cholic acid and on dihydroxycholenic acid (cf. Borsche and Todd, *Z. physiol. Chem.*, 1931, **197**, 173), have been formulated by Callow (*loc. cit.*) as (X; R = $C_5H_9O_2$, R′ = OH) for the α-acid and as (IX; R = $C_5H_9O_2$, R′ = OH) for the β-acid. The chemical and physical evidence with regard to these two acids is not in agreement throughout but a study of optical rotatory power (Table VII) and other facts makes it clear that these two formulations

TABLE VII.

Substance.	⊨ positions : literature.	⊨ positions : this paper.	Solvent.	$[M]_D$. Unsaturated acid.	$[M]_D$. Saturated acid.	Δ.	Refs.
α-Dihydroxycholadienic acid	7 : 8, 14 : 15	8 : 9, 14 : 15	Alcohol	−241	+220	+461	89, 96
β-Dihydroxycholadienic acid	8 : 9, 14 : 15	7 : 8, 14 : 15	Alcohol	+303	+220	− 83	89, 96

of Callow cannot be correct. The Δ value for α-dihydroxycholadienic acid is comparable with that for coprostadienol B and for *epi*coproergostadienol B (see Table V above); it must be reformulated, therefore, as

(IX; R = $C_5H_9O_2$, R′ = OH). The positively rotatory β-dihydroxycholadienic acid, on the other hand, is best formulated as (X; R = $C_5H_9O_2$, R′ = OH) in agreement with its reduction to β-*apo*cholic acid (see above).

Note on the use of Melting Point Differences in the Steroid Field.—If the same principles which lead to the additivity of the Δ values of molecular rotations were decisive in determining the temperature of fusion of organic compounds in general, and of steroids in particular, then a similar "Method of Differences" should operate in both cases. Broad generalisations relating melting point to structure are unknown, but it does seem possible to observe some regularities in the comparative melting points of a group of closely related substances such as the sterols. Thus for saturated sterols with rings A and B in the *cis*- or *trans*-position and with the 3-hydroxyl in the (β) or (α) orientation there are comparable differences in melting point between corresponding pairs of isomers in the cholestane and the β-sitostane series. The same applies in the ergostane series too, if the melting point of *epi*coproergostanol is taken as about 130°, but not if taken as 184° (the melting point of *u*-ergostanol). On the other hand the melting point for *u*-ergostanyl acetate is in agreement (as is its Δ value, see above) with that expected. These facts support the suggested inhomogeneity of *u*-ergostanol mentioned earlier in this paper.

An interesting fact about the melting points of simple sterols [of the 3(β)-ol type] is that all have higher melting points than their acetates except those which have been assigned a Δ^7 bond on optical rotation evidence in Parts I and II. When a Δ^7 bond is present in the molecules the acetates melt at a higher temperature than the parent sterols. This exceptional behaviour can be used in assigning a nuclear double bond position to the mixed stellasterols (see Bergmann and Stansbury, *J. Org. Chem.*, 1944, **9**, 281). These mixed sterols resist separation, and the nuclear double bond present cannot be hydrogenated catalytically. The Δ_1 value for the mixed sterols is − 23° and the Δ_2 value + 13°. An inspection of Table III, Part I, shows that these differences correspond only to a Δ^7 or Δ^{14} double bond position, the latter being eliminated on chemical grounds. The Δ^7 formulation is supported by the report that the melting point of the mixed acetates is greater than that of the parent sterols. It is predicted, therefore, that the mixed stellasterols all have a Δ^7 nuclear double bond and differ from each other only in side chain configuration and/or unsaturation—probably the latter.

Further points of interest are that the melting points of α-stenols of the 3(β)-*trans*-configuration are always

TABLE VIII.

Substance.	Solvent.	$[\alpha]_D$ (to nearest degree).	*c* (g. per 100 ml.).	*l* (dm.).	$[M]_D$.
Cholesterol	Acetone	−28	1·08	1	−116
	Acetone	−32	1·20	2	
	Alcohol	−27	1·17	1	−116
	Alcohol	−33	0·59	1	
	Alcohol	−30	1·00	2	
	Chloroform	−40 *	2·00	1, 2	−154
	Dioxan	−34	2·16	1	−131
Cholesteryl acetate	Acetone	−33	2·03	1	−141
	Acetone	−33	1·44	2	
	Acetone	−32	0·58	2	
	Alcohol	−30	0·72	1	−137
	Alcohol	−34	1·25	2	
	Chloroform	−44 *	2·00	1, 2	−188
	Dioxan	−38	1·93	1	−163
Cholestanol	Acetone	+29	1·65	2	+113
	Alcohol	+29	1·24	2	+113
	Chloroform	+23 *	2·00	1, 2	+ 89
	Dioxan	+25	1·78	1	+ 97
Cholestanyl acetate	Acetone	+19	0·72	2	+ 82
	Alcohol	+19	0·85	2	+ 82
	Chloroform	+14 *	2·00	1, 2	+ 60
	Dioxan	+15	2·08	1	+ 69
	Dioxan	+16	2·86	1	
Dehydro*iso*androsterone	Alcohol	+13	3·22	1	+ 37
	Alcohol	+12	1·29	2	
	Chloroform	+ 2 *	2·00	1, 2	+ 6
Dehydro*iso*androsterone acetate	Alcohol	+ 6	2·04	2	+ 20
	Chloroform	− 7 *	2·00	1, 2	− 23
*iso*Androsterone	Alcohol	+95	2·21	1	+276
	Alcohol	+95	0·88	2	
	Chloroform	+90	3·16	1	+261
	Chloroform	+90	1·26	2	
*iso*Androsterone acetate	Alcohol	+76	1·84	1	+249
	Alcohol	+74	0·74	2	
	Chloroform	+69	1·84	1	+229
	Chloroform	+68	0·74	2	

* These values are obtained from interpolation of a whole series of specific rotations obtained from *c* = 1 upwards. The change of rotation with concentration is small.

below those of any other isomer with nuclear unsaturation and that the Δ^4-3-ketones obtained by oxidising Δ^5-stenols are very much lower in melting point than the parent stenols, whereas when the double bond is in a different position in the nucleus no great change in melting point is observed on transformation to the ketone.

Although generalisations of this type can be made and are useful, there are many irregularities. A consideration of melting points can never yield the valuable results that optical rotatory power can, because the melting point is a function of both intra- and inter-molecular forces, whereas optical rotatory power is, in solution, mainly a manifestation of intramolecular forces only. Nevertheless a comparative examination of melting points should not be neglected in sterol work particularly when dealing with naturally occurring products.

Experimental.

All rotations were taken at 24° ± 2° and for the Na_D line, using a 1 or 2 dm. macro-tube. All substances examined were purified as carefully as possible before use and dried in a vacuum at 20° below their melting points. M. ps. are uncorrected. Cholesterol, m. p. 148·5°. Cholesteryl acetate, m. p. 114·5°. Cholestanol, m. p. 142·5°. Cholestanyl acetate (from cholesteryl acetate by catalytic reduction in acetic solution with platinum oxide catalyst), m. p. 110°. Dehydro-*iso*androsterone, m. p. 148°. Dehydro*iso*androsterone acetate, m. p. 172°. *iso*Androsterone, m. p. 174°. *iso*Androsterone acetate (from dehydro*iso*androsterone acetate by the method of Reichstein and Lardon, *Helv. Chim. Acta*, 1941, **24**, 955), m. p. 105°.

For the determinations of the rotations of cholesterol and its derivatives in alcohol and acetone, 2% by volume of chloroform was added to increase the solubility. Preliminary experiments showed that this small addition of chloroform had no appreciable influence on the optical rotations observed.

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