52. *Studies in the Sterol Group. Part XXXIX. The Structures of Ergosterol, Lumisterol, Pyrocalciferol, and* iso*Pyrocalciferol.*

By T. KENNEDY and F. S. SPRING.

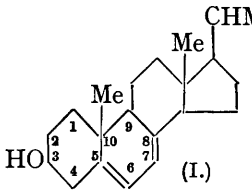
Pyrocalciferyl acetate yields a "*pinacol*" *diacetate* on irradiation with sunlight in the presence of eosin—a reaction also observed with ergosterol, dehydroergosterol, and dehydrolumisteryl acetate, but not with lumisterol or *iso*pyrocalciferyl acetate.

It is concluded that a positive " pinacol " reaction in the ergosterol series indicates a *trans*-orientation of the methyl group attached to C_{10} and the hydrogen attached to C_9, and that lumisterol, the primary photoisomeride of ergosterol (I), differs from the latter solely in the orientation around C_{10}.

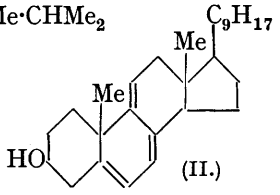
In Part XXXVII (Heilbron, Kennedy, Spring, and Swain, J., 1938, 869) it has been shown that the four stereoisomers ergosterol, lumisterol, pyrocalciferol, and *iso*pyrocalciferol may be represented as the four theoretically possible isomers of (I), in which the stereovariants are C_9 and C_{10}, and not as the four isomers in which C_3 and C_9 are the stereovariants. A possibility which has not been excluded is that in which C_3, C_9, and C_{10} are all variable centres of asymmetry; the orientation of each of these centres in ergosterol being arbitrarily indicated by +, the following representations are in agreement with the established chemical relationships:

	C_3.	C_9.	C_{10}.		C_3.	C_{10}.
Ergosterol	+	+	+	→ Dehydroergosterol	+	+
*iso*Pyrocalciferol	+	−	+			
Lumisterol	−	±(?)	−	→ Dehydrolumisterol	−	−
Pyrocalciferol	−	∓(?)	−			

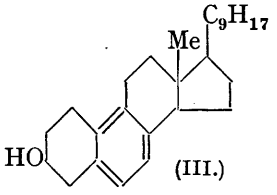
There is no evidence available to indicate whether the orientation around C_9 in lumisterol is the same as or different from that in ergosterol. These configurations account for the formation of dehydroergosterol (II) from either ergosterol or *iso*pyrocalciferol and of dehydrolumisterol (II) from either lumisterol or pyrocalciferol (Windaus and Dimroth,



(I.)



(II.)



(III.)

Ber., 1937, **70**, 376; Heilbron, Spring, and Stewart, J., 1935, 1221). Furthermore, *epi*-lumisterol would be represented by [C_3 +; C_9 ±(?); C_{10} −] and thus differs from either ergosterol or *iso*pyrocalciferol, a fact established by Heilbron, Kennedy, Spring, and Swain (*loc. cit.*).

The relationship of the four stereoisomers has been defined more closely by a study of " pinacol " formation in the ergosterol series. By irradiation of an alcoholic solution of ergosterol and eosin with sunlight in the absence of oxygen, Windaus and Borgeaud (*Annalen*, 1928, **460**, 235) obtained a sparingly soluble bimolecular oxidation product, " ergopinacol." The structure of " ergopinacol " has not been established; it is not a true pinacol, since it is completely resistant to lead tetra-acetate * and it readily forms a diacetate which in its turn is easily hydrolysed. A possible constitution for " ergopinacol " has been suggested by Inhoffen (*Naturwiss.*, 1937, **25**, 125). On thermal degradation " ergopinacol " evolves methane and gives *neo*ergosterol (III) (Inhoffen, *Annalen*, 1932, **497**, 130; Bonstedt, *Z. physiol. Chem.*, 1929, **185**, 165; Honigmann, *Annalen*, 1934, **511**, 292) in which ring B is aromatic, the two asymmetric centres C_9 and C_{10} being thereby removed. Lumisterol, in contrast to ergosterol, will not undergo photochemical oxidation to give a " pinacol " (Dimroth, *Ber.*, 1936, **69**, 1123), an observation which we have confirmed. Furthermore, it has been shown that dehydrolumisteryl acetate (Dimroth, *loc. cit.*) and dehydroergosterol (Windaus and Linsert, *Annalen*, 1928, **465**, 148) both readily yield bimolecular " pinacol " derivatives, the velocity of reaction in both cases being greater than in that of ergosterol. These facts strongly suggest that C_9 is the determining factor in " pinacol " formation. In order to test this theory, pyrocalciferyl and *iso*-pyrocalciferyl acetates have now been subjected to the " pinacol " reaction. If " pinacol " formation is governed by the orientation around C_9, since *iso*pyrocalciferol differs from

* This observation was made in these laboratories by Dr. K. M. Samant in 1933 in an attempt to prepare the then unknown ergostatrienone.

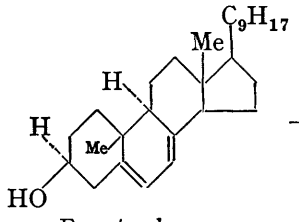
ergosterol solely in the orientation of this centre, it should not give a " pinacol." This we find to be the case, *iso*pyrocalciferyl acetate being unaffected by long irradiation with sunlight in the presence of eosin and absence of oxygen, there being no observable decoloration of the solution. In the second place, since lumisterol and pyrocalciferol differ solely in the orientation around C_9 and since the former does not give a " pinacol," the latter should do so. We find that pyrocalciferyl acetate * readily yields a " *pinacol* " *diacetate*, m. p. 196°, $[\alpha]_D^{20°}$ — 80°. Pyrolysis of this " pinacol " diacetate yields *neo*ergosteryl acetate identical with that directly obtained from " ergopinacol " diacetate. Thus a new experimental link has been forged between the two pairs ergosterol–*iso*pyrocalciferol on the one hand and lumisterol–pyrocalciferol on the other. It can now be concluded with a considerably higher degree of probability than heretofore that during the photochemical changes

ergosterol ⟶ lumisterol ⟶ tachysterol ⟶ calciferol

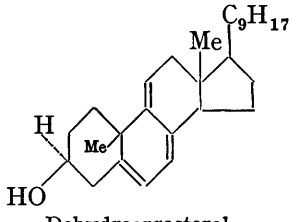
the orientation of the hydroxyl group remains unaltered. Whilst it was conceivable that, during the pyrolysis of calciferol, partial epimerisation at C_3 occurred with formation of pyrocalciferol, this is now highly improbable, since such an assumption would necessitate that, during the pyrolysis of pyrocalciferol " pinacol " diacetate, epimerisation of the same centre occurs in the reverse sense.

A consideration of " pinacol " formation now allows of a decision to be made concerning the relative orientation of C_9 in ergosterol and lumisterol. The inability of lumisterol (I) to form a pinacol, together with the positive " pinacol " reaction of ergosterol (I) and the two dehydrosterols dehydrolumisterol and dehydroergosterol (II) in which the steric effect of the C_9-hydrogen has been removed, suggests that the C_{10}-methyl group and the C_9-hydrogen atom are *cis*-oriented in lumisterol and *trans*-oriented in ergosterol. This conclusion requires that these centres be *cis*-oriented in *iso*pyrocalciferol and *trans*-oriented in pyrocalciferol, these representations being completely in harmony with the established chemical relationships and the " pinacol " rule. Employing for ergosterol a *cis*-configuration for the C_3-hydroxyl group relative to the C_{10}-methyl group, we arrive at the following configurations :

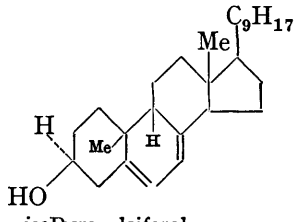
Pinacol formation.		C_3.	C_{10}.	C_9.		C_3.	C_{10}.
+	Ergosterol	+	+	−	} → Dehydroergosterol	+	+
−	*iso*Pyrocalciferol	+	+	+			
−	Lumisterol	+	−	−	} → Dehydrolumisterol	+	−
+	Pyrocalciferol.....	+	−	+			



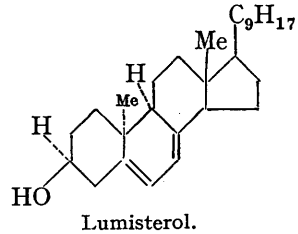
Ergosterol.



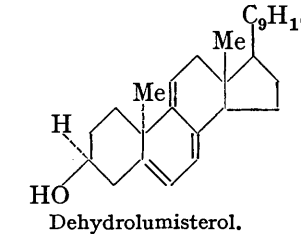
Dehydroergosterol.



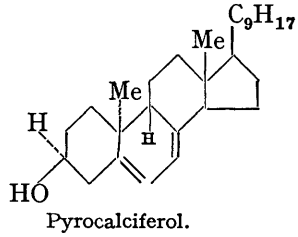
*iso*Pyrocalciferol.



Lumisterol.



Dehydrolumisterol.



Pyrocalciferol.

* Using pyrocalciferol, Dimroth (*loc. cit.*) failed to observe " pinacol " formation. This result is probably due to the relatively great solubility of the free " pinacol."

Experimental.

Pyrocalciferol " Pinacol " Diacetate.—Pyrocalciferyl dinitrobenzoate was prepared by Busse's method (*Z. physiol. Chem.*, 1933, **214**, 211) and had m. p. 170—171°, $[\alpha]_D^{20°}$ + 194° (l = 1, c = 0·1 in chloroform). Hydrolysis of the ester gave pyrocalciferol, m. p. 94—95°, $[\alpha]_D^{20°}$ + 508° (l = 1, c = 0·03 in alcohol), acetylation of which gave pyrocalciferyl acetate, m. p. 81—82°, $[\alpha]_D^{20°}$ + 407° (l = 1, c = 0·09 in chloroform). A solution of pyrocalciferyl acetate (1·4 g.) and eosin (1·4 g.) in alcohol (110 c.c.) was boiled to expel the air, and the flask sealed while hot. After 3 days' exposure to sunlight, a crystalline mass had separated. The irradiation was continued with intermittent shaking for 2 weeks; the colour of the solution had then considerably diminished. The separated mass was collected and crystallised from alcohol, from which *pyrocalciferol " pinacol " diacetate* separated in long needles, m. p. 196°, $[\alpha]_D^{20°}$ — 80° (l = 1, c = 0·06 in chloroform) (yield, 10%) (Found: C, 82·0; H, 10·4. $C_{60}H_{90}O_4$ requires C, 82·3; H, 10·4%).

neo*Ergosteryl Acetate.*—Pyrocalciferol " pinacol " diacetate (120 mg.) was heated for 2 hours at 180—190°/0·1 mm. The pressure was then reduced to 0·0001 mm., and the temperature maintained at 180—190°. The distillate was crystallised from acetone, from which *neo*-ergosteryl acetate separated in needles, m. p. 121—122° (Found: C, 82·4; H, 9·8. Calc. for $C_{29}H_{42}O_2$: C, 82·35; H, 10·05%). No depression of the m. p. occurred when this specimen was mixed with authentic *neo*ergosteryl acetate prepared in the same manner from " ergopinacol " diacetate.

The University, Manchester. [*Received, December 10th*, 1938.]