XVIII. THE COLOUR REACTIONS AND ABSORPTION SPECTRA OF STEROLS IN RELATION TO STRUCTURE.

BY ISIDOR MORRIS HEILBRON AND FRANK STUART SPRING.

From the Department of Organic Chemistry, Liverpool University.

(Received November 9th, 1929.)

THE production of an immediate carmine red colour with an aqueous solution of trichloroacetic acid has been shown, in all probability, to be dependent upon the presence of a $\Delta^{1:13}$ (or $\Delta^{1:2}$) ethenoid linkage in the sterol ring system [Rosenheim, 1929]. In accordance with this cholesterolene [Bose and Doran, 1929], ψ -cholestene, *allocholesterol* [Windaus, 1927] and ergosterol [Heilbron and Sexton, 1929] all give the reaction, the latter being further characterised by the fact that the red coloration passes rapidly to blue.

Another reaction which has been stated to be specific for the detection of ergosterol or its derivatives (dehydroergosterol and *isoergosteryl acetate*) is the green coloration produced by the Tortelli-Jaffé reaction [Häussler and Brauchli, 1929]. Employing this method the colour only develops slowly (about 10 minutes) and is only just discernible with 0.5 mg. ergosterol. The authors point out, however, that they have purposely not altered the standardised method of carrying out the reaction. We find that by employing the following modification the reaction can be rendered both instantaneous and more sensitive. A crystal of ergosterol is dissolved in glacial acetic acid (5 cc.) and 1 cc. of a 2 % solution of bromine in chloroform is introduced down the side of the tube by means of a pipette; a green ring appears at the surface of contact of the two solutions. Using these conditions, 0.02 mg. ergosterol can be immediately detected. The reaction is of peculiar importance, however, as the appearance of colour occurs, as shown in Table I, in hydrogenated derivatives of ergosterol which do not respond to either the Rosenheim or SbCl_3 reagent.

Whereas the compounds responding to the Rosenheim reaction are characterised by the presence of the $\Delta^{1:13}$ (or $\Delta^{1:2}$) double bond, it is evident that the appearance of colour in the bromine reaction is dependent upon some other ethenoid linkage. An insight into the position of this ethenoid linkage is gained in the first place from the non-reactivity of cholesterolene towards this reagent, and secondly from the positive reaction given by ergosterol. According to Bose and Doran [1929] cholesterolene may be represented as shown in formula I, whilst ergosterol is formulated as II [Heilbron and Sexton,

1929]. It follows from this that the bromine reaction is dependent upon the presence of the inert $\Delta^{10:19}$ (or $\Delta^{10:11}$) ethenoid linkage not present in cholesterolene. Thus both α - and β -dihydroergosterol to which the formulae III and IV have been respectively ascribed [Heilbron, Johnstone and Spring, 1929] fail to give the Rosenheim reaction, but react positively towards bromine. Again both α - and β -isosterol (formula V) [Heilbron and Spring, 1929] show, as anticipated, both the Rosenheim and the bromine reaction. The same applies to dehydroergosterol ($C_{27}H_{40}O$, F_4); the presence of the "inert" linkage in this compound is demonstrated by the fact that hydrogenation leads to α -ergosterol [Windaus and Linsert, 1928].

Table I.

Compound	Rosenheim reagent (trichloroacetic acid)	$SbCl_3$	Bromine	Absorption spectra
Ergosterol	Pink $\rightarrow$ blue	Red	Positive	Bands at 260, 270, 281, 293-5 μ
α -Dihydroergosterol	Negative	Negative	Positive	No selective absorption
β -Dihydroergosterol	Negative	Negative	Positive	No selective absorption
α -isosterol	Pink	Yellow	Positive	Band at 247 μ
β -isosterol	Pink	Yellow	Positive	Band at 247 μ
Dihydroisosterol	Pink	Yellow	Positive	Band at 247 μ
Dehydroergosterol	Pink $\rightarrow$ green	Blue	Positive	Band at 313, 326, 344 μ
α -Ergosterol	Negative	Negative	Positive	No selective absorption
β -Ergosterol	Negative	Negative	Negative	No selective absorption
<i>allo</i> - α -Ergosterol	Negative	Negative	Negative	No selective absorption
Cholesterol	Negative	Negative	Negative	No selective absorption
Cholesterolene	Carmine-red	Carmine	Negative	Bands at 294, 304, 321 μ
Cholestene	Negative	Negative	Negative	No selective absorption
ψ -Cholestene	Carmine-red	Brown-red	Negative	No selective absorption
<i>allo</i> -Cholesterol	Yellow-orange	Brown-red	Negative	No selective absorption
β -Cholesterol	Carmine-red	Red-brown	Negative	No selective absorption

The most important evidence regarding the specificity of the bromine reaction in relation to the "inert" ethenoid linkage is obtained from a comparison of α -ergosterol (tetrahydroergosterol) to which we ascribe the structure shown in formula VI, with its position isomer β -ergosterol [Reindel, Walter and Rauch, 1927]. Whereas the former compound fails to give, as anticipated, either the Rosenheim or the parallel antimony trichloride reaction, it readily shows the bromine coloration. On the other hand β -ergosterol gives no colour with any of the reagents. That the $\Delta^{10:19}$ double bond is absent from this compound is evident from the fact that it is readily hydrogenated to the fully saturated *allo*- α -ergostanol (hexahydroergosterol) which also fails to give colour reactions.

As shown in Table I no cholesterol derivative gives a positive reaction with bromine, a fact again explicable by the ease of complete hydrogenation of all such compounds and consequent absence of any "inert" linkage.

Selective absorption. Heilbron, Morton and Sexton [1928] have suggested that the power of selectively absorbing in the ultra-violet is only shown by sterol derivatives containing at least two double bonds in the molecule. It now appears that this statement can be further qualified as, of the sterol derivatives so far examined, only those show selective absorption which contain not only two double bonds but give positive colour reactions with trichloroacetic acid or antimony trichloride, *i.e.* which contain the $\Delta^{1:13}$ (or $\Delta^{1:2}$) ethenoid linkage. Thus ergosterol, α - and β -isoergosterol, dihydro-isoergosterol [Heilbron and Spring, 1929], dehydroergosterol and cholesterolene all give coloured solutions in presence of the Rosenheim reagent or SbCl_3 , and all show well-defined bands in the ultra-violet. On the other hand although both α - and β -dihydroergosterol contain two double bonds in the molecule, the potent $\Delta^{1:13}$ linkage is absent and only general absorption is shown.

SUMMARY.

1. The Tortelli-Jaffé reaction has been modified so as to render it more sensitive. The reagent appears to be specific for sterol derivatives containing an "inert" (as applied to hydrogenation) linkage, possibly in position $\Delta^{10:19}$.

2. Selective absorption of sterols is conditioned by the presence of two ethenoid linkages in the molecule, one of which must apparently be in the $\Delta^{1:13}$ (or $\Delta^{1:2}$) position.

REFERENCES.

- Bose and Doran (1929). *J. Chem. Soc.* 2244.
- Häussler and Brauchli (1929). *Helv. Chim. Acta*, **12**, 187.
- Heilbron, Johnstone and Spring (1929). *J. Chem. Soc.* 2248.
- Morton and Sexton (1928). *J. Chem. Soc.* 47.
- and Sexton (1929). *J. Chem. Soc.* 921.
- and Spring (1929). *J. Chem. Soc.* 2807.
- Reindel, Walter and Rauch (1927). *Liebig's Ann.* **452**, 34.
- Rosenheim (1929). *Biochem. J.* **23**, 49.
- Windaus (1927). *Liebig's Ann.* **453**, 101.
- and Linsert (1928). *Liebig's Ann.* **465**, 148.