CXLII. THE IODINE VALUES OF SOME STEROLS BY DAM'S METHOD.

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A NUMBER of investigators have pointed out that abnormal iodine values are obtained for sterols and their derivatives when the widely used methods of Wijs or Hübl are employed. Smedley-MacLean and Thomas [1920] (see also Daubney and Smedley-MacLean [1927]) made special reference to the variable values obtained for the unsaponifiable matter of yeast fat.

In the extraction of unsaponifiable fractions, often largely sterol, in this laboratory, a method for iodine values which would give trustworthy results would be of great value. It seemed that the method of Dam [1924] might provide this. Dam's reagent was made up and tested by a series of experiments with cholesterol. Ordinarily 0.25 g. of cholesterol in 10 cc. of chloroform was used, but the method proved trustworthy for 0.025 g. or less, provided that a suitable excess of reagent was used.

Some estimations of the iodine value of pure ergosterol, and also of the sterol content of the unsaponifiable matter of yeast fat were then made. The results were practically the same as those obtained by previous observers.

Ergosterol gave values ranging from 264 to 293 for different samples. On considering these high values there seemed a possibility that they might be accounted for by a fourth double bond, since in this case the theoretical number would be 266, instead of 199 as for three double bonds. Alternatively, the conclusion would be that there is a marked substitution in the ergosterol molecule. In order to throw some light on the matter an investigation of the iodine values of other sterols was undertaken. The results are shown in full in Table I.

The theoretical iodine value has been calculated for each compound, and, in general, it is found that sterols with one double bond give values approximating most nearly to the calculated result. Also, where there is only one double bond the time of contact with the reagent does not seem to be of great importance.

In the case of cholestenone, which has a ketonic linkage in the molecule, the iodine value is very low, being about half the theoretical value if the solution is treated immediately and even less when the contact with the

reagent is longer. A similar peculiarity is shown by oxycholesterylene which has two double bonds with a ketonic linkage. Stigmasterol with two double bonds gives an approximately correct result, but cholesterylene gives a value of 75 instead of 138. Mauthner and Suida [1896] report similar figures, using Wijs's method. They also state that cholestene with one double bond, and cholesterylene with two give almost identical results.

Table I. *Iodine values of sterols by Dam's method.*

Compound	No. of ethylenic bonds	Amount taken (g.)	Time of contact (mins.)	Iodine value	Theoretical iodine value
Cholesterol	1	0.25	5	65.1	65.5
"	1	0.25	15	65.3	65.5
"	1	0.25	30	67.1	65.5
Cholestenone	1 + C=O	0.25	0	33.0	66.1
"	1 + C=O	0.25	5	31.0	66.1
"	1 + C=O	0.25	15	12.0	66.1
Cholesteryl chloride	1	0.25	0 or 5	62.6	62.7
Cholestene	1	0.25	0	71.9	68.8
Cholesterylene	2	0.0125	0	75.0	138.0
Oxycholesterylene	2 + C=O	0.0125	0	85.0	133.0
"	2 + C=O	0.0125	5	73.0	133.0
Phytosterol	1	0.25	0	66.5	65.5
"	1	0.25	5	70.0	65.5
Sitosterol	1	0.025	0	70.5	65.5
Lupeol	1	0.025	0	68.6	65.5 (?)
"	1	0.025	5	72.3	65.5
Stigmasterol	2	0.0125	2	102.6	119.0
"	2	0.0125	5	126.0	11.9
Ergosterol (a)	3 (or 4)	0.05	0	289.0	199.0 (or 266.0?)
" (a)	3 (or 4)	0.07	5	268.4	199.0
" (a)	3 (or 4)	0.05	15	400.0	199.0
" (b)	3 (or 4)	0.05	0	264.0	199.0
" (b)	3 (or 4)	0.05	5	282.0	199.0
" (c)	3 (or 4)	0.005	0	293.0	199.0
allo-Cholesterol	1	0.025	0	61.8	65.5
allo-Sitosterol	1	0.025	0	71.5	65.5
Coprosterol (a)	0	0.02	0	9.0	—
" (b)	0	0.02	0	7.5	—
α-Cholesterol oxide	0	0.025	0 or 5	2.4	—
β-Amyrol	0	0.0107	0	90.2	—
"	0	0.0182	5	90.6	—

When less than 0.05 g. was taken a micro-method was used.

The iodine value of ergosterol was determined on three different samples: (a) as supplied by British Drug Houses, Ltd., (b) as extracted from distillery yeast, and (c) a specially pure yeast ergosterol prepared by Dr Rosenheim. All the values were about 266 or higher, and it was found that the time of contact very definitely affected the values, though with the most rapid titration none lower than 264 could be obtained. A similar result is reported by Smedley-MacLean [1928], who ascribes the high iodine value to a peculiarity of structure rather than to the presence of additional ethenoid linkages.

It seems, however, that structural differences within the molecule do not generally affect the iodine value of a sterol, as is shown by *allo*-cholesterol and *allo*-sitosterol, which give practically the same iodine values as their isomers. This makes it the more difficult to explain the high iodine value of ergosterol. The only other case of extraordinary absorption is that of β-amylol which,

though a saturated compound, gives an iodine value of 90. The other saturated compounds, coprosterol and α -cholesterol oxide, give almost negligible iodine values.

Dam [1925] reports also a modification of Wijs's method, in which, by using a suitable excess of the reagent, the time can be reduced to one hour. Using this method in a series of experiments with cholesterol a low value of about 44 was obtained. The result was the same whether the time of contact was one, two, or three hours. Cholestenone and cholesterylene give similarly low values.

SUMMARY.

By the pyridine-sulphate-dibromide method of Rosenmund and Kuhnenn [1923] as described by Dam [1925] certain sterols yield abnormal values.

Ergosterol shows a high iodine value which is probably due to substitution and not to the presence of a fourth double bond.

The presence of a ketonic group in the molecule caused a low iodine value in the substances tested.

Structural differences within the molecule of the type represented by *allo*-cholesterol do not cause variations in the iodine values of sterols of the same empirical formula.

Except in the case of ergosterol the duration of the reaction does not greatly affect the iodine value.

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