XXXV. OBSERVATIONS ON ABNORMAL IODINE VALUES, WITH SPECIAL REFERENCE TO THE STEROLS AND RESINS.

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WHILE investigating the nature of yeast fat [Smedley MacLean and Thomas, 1920] we noticed that the sterol of yeast fat exhibits a marked difference in its iodine value when this is determined respectively by the Wijs and Hübl methods. This abnormal behaviour was described by Werner [1911] for cholesterol and sitosterol and is referred to in the text-book of Lewkowitsch [1913]. It seemed to us desirable to try and ascertain how far this property was peculiar to the sterols and whether it could be shown to be due to any definite grouping of atoms.

The iodine value of cholesterol was first determined by Lewkowitsch [1892] by Hübl's method; he obtained the number 65·8, agreeing approximately with that required by the presence of one double bond ($C_{27}H_{46}O \cdot H_2O$ requires 62·8). Using the same method, Werner found for sitosterol values ranging from 41 to 76 but after only half an hour's contact with the Wijs solution the iodine number had reached 135.

The sterols examined in the present investigation were cholesterol, brassicasterol, yeast sterol (ergosterol), dihydrocholesterol and coprosterol; of these the last two alone show only slight absorption of iodine with either the Wijs or Hübl solution.

Resins also show great variations in iodine values according to the conditions of the determination, the Wijs values reaching higher figures than the Hübl ones. McIlhiney [1894, 1902] showed that the Hübl iodine number of the resins was considerably affected by the time of absorption and by the excess of halogen used. Smetham and Dodd [1900] found that the Wijs numbers were similarly influenced. American colophony and the abietic acid obtained from it also give abnormally high Wijs values and will be discussed later.

The naphthenic acids constitute another class of substances which are described as showing differences in the iodine values determined respectively by the Hübl and Wijs methods; the iodine values are, however, low. The material used consisted of residues from natural petroleum and there is insufficient evidence as to the nature of the individual substances contained in them. Marcussohn [1905, 1907] and others have used the similar behaviour

of the naphthenic acids and cholesterol when treated with iodine reagents as an argument for the animal origin of petroleum, these acids being regarded as derived from cholesterol.

Wijs' reagent consists essentially of a solution of iodine monochloride in glacial acetic acid; Hübl's reagent is made by mixing solutions of mercuric chloride and iodine, each in 95 % spirit, and in this iodine monochloride is considered to be the active substance. We have, therefore, a solution in the one case of iodine monochloride in alcohol and in the other, of the same compound in acetic acid; with those unsaturated compounds, in which addition of halogen is the only reaction, both appear to act similarly as iodinating agents.

A survey of the literature shows that the monochloride of iodine has been used before as an iodinating agent. Michael and Norton [1878] used this for the preparation of mono-, di- and tri-derivatives of phenol, aniline, and para-acetanilide, and Willgerodt [1901] for the iodination of the nitranilines, the degree of the iodination increasing with the relative amount of iodine chloride used. Sernow [1903] has also used this reagent for the iodination of acid chlorides.

Iodine monochloride, therefore, especially in acetic acid solution produces substitution in certain aromatic compounds and one product of this reaction is an iodo-derivative, whilst hydrochloric acid must also be formed. The presence of the acetic acid in the Wijs solution favours substitution. It is possible that the mercuric salt in the Hübl reagent may have a special influence in diminishing substitution.

The sterols and resin acids show in a well-marked degree the property of discriminating between the action of these two iodine reagents; we also examined a considerable number of substances of known structure in order to determine whether any special atomic groupings could be connected with this property. The iodine values determined by the Hübl and the Wijs methods respectively are shown in the accompanying table and the results are discussed below. Where substitution occurs, the iodine numbers vary within wide limits, depending on the time of contact and the proportions of the reacting substances, so that for such substances, there is no one definite iodine value.

A survey of the figures on pp. 321, 322 shows that the Hübl values are markedly greater than those obtained by the Wijs method in the case of phenol and to a much less extent in the case of naphthol and the cinnamyl series, alcohol, aldehyde and acid. The iodine values of the phenols were investigated first by Fahrion [1901]. The Hübl value is much higher than that determined by the Wijs reagent; the number of halogen atoms absorbed in the two cases being respectively 3.2 and 2.0. Wake and Ingle [1908] suggest that phenol reacts as a cyclic ketone

	Formula	Hübl iodine value	Wijs iodine value	No. I atoms added by 1 mol. substance	
				Hübl	Wijs
HYDROCARBONS:					
Benzene	C_6H_6	0	0	0	0
Naphthalene	$C_{10}H_8$	0	0	0	0
Fluorene	$C_{13}H_{10}$	$\left\{ \begin{array}{l} 5.7 \\ 6.2 \end{array} \right.$	$\left\{ \begin{array}{l} 18.2 \\ 20.5 \end{array} \right.$	0.1	0.2
Anthracene	$C_{14}H_{10}$	171 to 237	149 to 256	to 3.5	to 3.8
Phenanthrene	$C_{14}H_{10}$	$\left\{ \begin{array}{l} 10.8 \\ 11.5 \end{array} \right.$	From 20.0 ¹ to 100.9	0.1	0.2 to 1.3
Retene	$C_{18}H_{18}$	$\left\{ \begin{array}{l} 13.4 \\ 14.4 \end{array} \right.$	$\left\{ \begin{array}{l} 116.1 \\ 115.0 \end{array} \right.$	0.2	2.1
(1-methyl-7-isopropylphenanthrene)					
ALCOHOLS:					
Normal primary alcohols (ethyl to octyl)					
		0	0	0	0
Allyl alcohol	C_3H_6O	$\left\{ \begin{array}{l} 414.3 \\ 425.5 \end{array} \right.$	$\left\{ \begin{array}{l} 435.0 \\ 444.1 \end{array} \right.$	1.95	2.03
$CH_2:CH.CH_2OH$					
Cinnamyl alcohol	$C_9H_{10}O$	$\left\{ \begin{array}{l} 18.2 \\ 18.0 \end{array} \right.$	$\left\{ \begin{array}{l} 7.6 \\ 9.2 \end{array} \right.$	0.2	0.1
$C_6H_5.CH:CH.CH_2OH$					
Terpene alcohols	$C_{10}H_{18}O$	263.6	244.3 ²	—	3.0
Geraniol (B.P. 125–8°, 20 mm.)		264.0	277.4 ³	3.2	3.4
$(CH_3)_2C:CH.CH_2.CH_2.C(CH_3):CH.CH_2OH$					
Linalool (B.P. 124°, 60 mm.)		367.8	217.6	4.5	2.6
$(CH_3)_2.C:CH.CH_2.CH_2.C.(CH_3)(OH).CH:CH_2$		374.0	216.0		
Terpineol		182.3	182.7	2.2	2.2
<div>$\begin{array}{c} CH-CH_3 \\ \parallel \\ CH_3.C \quad CH.CO.H(CH_3)_2 \\ \diagup \quad \diagdown \\ CH_2-CH_2 \end{array}$</div>					
Dihydrocarveol		$\left\{ \begin{array}{l} 174.6 \\ 180.8 \end{array} \right.$	$\left\{ \begin{array}{l} 182.3 \\ 184.3 \end{array} \right.$	2.2	2.2
<div>$\begin{array}{c} CHOH.CH_2 \quad CH_3 \\ \diagup \quad \diagdown \\ CH_3.CH \quad CH.C \\ \diagdown \quad \diagup \\ CH_2.CH_2 \quad CH_3 \end{array}$</div>					
Menthol		0	0	0	0
<div>$\begin{array}{c} CH_2-CHOH \\ \diagup \quad \diagdown \\ CH_3CH \quad CH.CH(CH_3)_2 \\ \diagdown \quad \diagup \\ CH_2-CH_2 \end{array}$</div>					
PHENOLS:					
Phenol	C_6H_5OH	$\left\{ \begin{array}{l} 473.3 \\ 484.8 \end{array} \right.$	$\left\{ \begin{array}{l} 274.8 \\ 274.6 \end{array} \right.$	3.5	2.0
α -Naphthol	$C_{10}H_7OH$	277.9	274.6	3.2	3.1
β -Naphthol	$C_{10}H_7OH$	$\left\{ \begin{array}{l} 177.9 \\ 184.0 \end{array} \right.$	$\left\{ \begin{array}{l} 174.3 \\ 183.1 \end{array} \right.$	2.1	2.1

¹ The values obtained varied from 26 to 101, the number increasing especially with the proportion and concentration of the iodine solution and with the temperature.

² With 15 cc. Wijs solution.

³ With 30 cc. Wijs solution.

				No. I atoms added by 1 mol. substance	
Formula		Hübl iodine value	Wijs iodine value	Hübl	Wijs
ALDEHYDES:					
Butyraldehyde	 C ₄ H ₈ O	0	up to 339	0	2.0
Isovaleraldehyde	 C ₅ H ₁₀ O	{ 26.56	263.9	0.2	1.8
		{ 29.12	263.1		
Oenanthol	 C ₇ H ₁₄ O	{ 22.1	198.5	0.2	1.8
		{ 24.1	198.9		
Benzaldehyde	 C ₇ H ₆ O	1.3	1.4	0	0
Crotonaldehyde	 C ₄ H ₆ O	{ 334.3	173.6		
CH ₃ .CH:CH.CHO		{ 334.2	171.8	1.9	1.0
Citral	 C ₁₀ H ₁₆ O	{ 268.1	268.4		
CH ₃ .C=CH.CH ₂ .CH ₂ .C=CH.CHO		{ 281.0	287.8	3.4	3.4
CH ₃	CH ₃				
Cinnamaldehyde	 C ₉ H ₈ O	143.3	85	1.5	0.9
C ₆ H ₅ CH:CH.CHO		156.7	81		
KETONES:					
Acetone	 CH ₃ CO.CH ₃	{ 21.1	164.6		
		{ 24.1	165.0	0.1	0.8
Acetophenone	 C ₆ H ₅ .CO.CH ₃	0	204.2 ¹	0	1.9
QUINONES:					
Anthraquinone	...	0	0	0	0
Phenanthraquinone		0	0	0	0
STEROLS:					
Cholesterol	 C ₂₇ H ₄₆ O.H ₂ O	{ 72.8	145.7 ²	2.3	5.0
		{ 72.1	164.3		
Dihydrocholesterol	 C ₂₇ H ₄₈ O.H ₂ O	2.6	3.0	0.1	0.1
			2.6		
Coprosterol	 C ₂₇ H ₄₈ O	{ 23.8 ³	33.2 ³	0.7	1.0
		{ 9.3 ⁴	18.2 ⁴	0.3	0.55
Brassicasterol	 C ₂₈ H ₄₆ O.H ₂ O	118.6	282.2	3.9	9.2
Yeast sterol (ergosterol)	 C ₂₇ H ₄₂ O.H ₂ O	{ 207.2	339.5	6.5	—
		{ 177.1	337.8	5.6	10.7
ACIDS:					
Crotonic	 CH ₃ CH:CH.COOH	{ 25.0 ⁵	—	0.09	—
		{ 25.9			
Cinnamic	 C ₆ H ₅ CH:CH.COOH	{ 12.75	6.2	0.08	0.03
		{ 4.7 ⁵			
Abietic	 C ₂₀ H ₃₀ O ₂	{ 156.6	239.4		
		{ 162.1	242.5	3.8	5.7
Dihydroabietic	 C ₂₀ H ₃₂ O ₂	75.1	237.4		
			231.8	1.8	5.6

¹ See p. 323.² Values vary with iodine concentration and temperature, see p. 326.³ Left for 24 hours at laboratory temperature in summer.⁴ In winter.⁵ Lewkowitsch [1913].

It will be shown later that the Wijs reagent favours the change from the ketonic to the enolic form and it is noteworthy that where the reverse change may be involved Hübl's solution gives the larger numbers.

The naphthols show little difference between the actions of the two iodine reagents, but the number of halogen atoms absorbed is greater with α -naphthol than with the β -compound. If the halogen absorption is conditioned by a

preliminary change from the enolic to the ketonic form, it would follow that (1) the Wijs reagent has a more potent influence in maintaining the enolic form of phenol than that of the naphthols and (2) that α -naphthol reacts more readily as a cyclic ketone than does the β -compound.

The abnormalities shown by the terpene alcohols may be explained if two facts are taken into account: (1) the nature of the groups attached to the double bonds and (2) the action of the acetic acid in the Wijs solution. In geraniol the double bond is in the α , β -position to the primary alcohol group, and the effect of the two alkyl groups attached to the β carbon and of the CH_2OH group attached to the α carbon atom is to diminish the iodine absorption, just as in cinnamyl alcohol the position of the phenyl and CH_2OH groups prevents the iodine absorption (cp. p. 324). In geraniol, therefore, both Hübl and Wijs values are low and, as might be expected, they are considerably influenced by the concentration of iodine. With linalool the Hübl value is slightly greater than normal, the Wijs value low. Zeitschel [1906] showed, however, that the effect of glacial acetic acid on linalool in the presence of certain chlorides was to convert linalool into a mixture of geraniol and nerol. The conditions to produce isomerisation of the linalool are present in the Wijs solution and may account for the low Wijs values obtained.

In certain cases, the Wijs values are somewhat higher than those obtained with the Hübl reagent, as for instance in the condensed ring hydrocarbons, naphthalene, fluorene and anthracene, but here the differences are small, corresponding to the absorption of from 0.1 to 0.2 of an iodine atom. It is possible also that some of these slight differences may be due to the presence of small amounts of impurities in the substances examined.

Four groups of substances however discriminate sharply between these two reagents giving much higher Wijs values.

- (1) Carbonyl compounds (fatty aldehydes and ketones).
- (2) Hydrocarbons containing the phenanthrene nucleus.
- (3) The sterols with the exception of the reduced sterols, coprosterol and dihydrocholesterol.
- (4) The resinic acids, abietic acid and to a greater extent its reduced derivative, dihydroabietic acid.

(1) *Carbonyl compounds.*

With a considerable excess of Wijs reagent, the lower fatty aldehydes give iodine numbers corresponding approximately to the absorption of two iodine atoms by one molecule of the substance, the Hübl values being much lower. The proportion of iodine absorbed from Wijs solution appears to be greatly affected by the relative concentration of aldehyde and iodine: the blue starch iodine colour returns very rapidly after the end-point is reached. Acetone and acetophenone also discriminate between the two reagents.

The Hübl value of acetophenone was found to be nil—the Wijs value anything from 10 to 202, the latter number corresponding to the absorption

of two halogen atoms. The great variation in the Wijs values obtained was due to differences in the laboratory temperature.

Thus at -10° the iodine value was 11.1; at 13° 151.9; at 26° 204.2; and at 36° 199.4. In an experiment left for the same time over night in the laboratory a value of only 34.2 was found. The night temperature was probably lower than 13° though the day temperature was 20° . Probably a temperature of over 20° is necessary to ensure that complete enolisation is produced by the Wijs solution.

Lapworth [1904] in his work on the halogenation of the ketones, showed that the addition of halogen to the carbonyl group is influenced by the presence of acids; their effect is to catalyse the change from the ketonic to the enolic form which was shown by Lapworth to precede addition. It appeared possible therefore that the glacial acetic acid used as the solvent for the iodine monochloride in the Wijs solution might help the enolisation of the ketone or aldehyde and so determine the absorption of iodine. We tried to test this by adding an equal volume of glacial acetic acid to the Hübl reagent, but under these conditions much of the acid is converted to ethyl acetate by the alcohol of the Hübl solution. The addition of an equal volume of concentrated hydrochloric acid (sp. gr. 1.16) to the Hübl reagent produced a great increase in the iodine value, but the blue starch-iodine colour returned so rapidly that the end-point was not very satisfactory.

Name	Wijs iodine value	Hübl iodine value	Hübl and HCl iodine value
Butyraldehyde ...	339	17.9	192.6
Oenanthal ...	183.0	40.4	125.7

The behaviour of benzaldehyde is in accordance with this explanation. Here, there is no possibility of enolisation and no iodine is absorbed. It seems probable therefore that the Wijs reagent acts by increasing the enolisation of the aldehyde or ketone and that the amount of halogenation depends on the degree of enolisation. It may well be that the glacial acetic acid used as solvent influences the enolisation.

The α , β -unsaturated aldehydes show an entirely different behaviour. In the crotonic and cinnamyl series (alcohol, aldehyde and acid) the Hübl values are considerably greater than those obtained with the Wijs reagent; cinnamyl alcohol and cinnamic and crotonic acids give very small values with both reagents but the corresponding aldehydes absorb approximately two iodine atoms when Hübl's reagent is used. In the following table, the figures represent the number of iodine atoms absorbed per molecule of substances.

Substance	Hübl	Wijs	Substance	Hübl	Wijs
Allyl alcohol ...	1.9	2.0	Cinnamyl alcohol ...	0.2	0.1
$\text{CH}_2 : \text{CH} \cdot \text{CH}_2\text{OH}$			$\text{C}_6\text{H}_5\text{CH} : \text{CH} \cdot \text{CH}_2\text{OH}$		
Crotonaldehyde ...	2.0	1.0	Cinnamyl aldehyde	1.3	.85
$\text{CH}_3\text{CH} : \text{CH} \cdot \text{CHO}$			$\text{C}_6\text{H}_5\text{CH} : \text{CH} \cdot \text{CHO}$	1.5	.88
Crotonic acid ...	0.1	—	Cinnamic acid ...	0.1	.05
$\text{CH}_3\text{CH} : \text{CH} \cdot \text{COOH}$			$\text{C}_6\text{H}_5\text{CH} : \text{CH} \cdot \text{COOH}$		

If the assumption made in the case of the saturated carbonyl compounds

is correct, namely that the enolisation of these compounds is increased by the acid of the Wijs solution, we must infer that in the conjugated aldehydes there is little tendency to enolisation. The greater protection against halogen addition afforded by the alcohol and carboxyl groups suggests that the aldehyde group when conjugated with an ethenoid group is itself concerned in the addition of halogen, which probably takes place in the following manner:

The product formed by the action of Hübl solution on crotonaldehyde is an aldehyde; it contains iodine but very little chlorine and attacks the eyes. We have not yet succeeded in isolating it in a sufficiently pure condition for analysis.

The absorption of iodine by the unsaturated aldehydes is a gradual process, as will be seen from the following figures:

No. of hours	...	...	2	4	6	18	24	48	168	Theory for one double bond
Iodine value of										
(a) Crotonaldehyde	...	...	44	66	107	140	172	174	202	350
(b) Cinnamaldehyde	...	...	39	52	64	84	96	117	103	190.5
(c) Citral	...	...	169	241	257	283	304	374	—	167

In citral, the 5, 6 double bond seems to react normally: the conjugated carbonyl and 1, 2 ethenoid linkages show a gradual absorption similar to that of croton and cinnamic aldehydes. After deducting the value for two iodine atoms absorbed by the 5, 6 double bond, 1.4 iodine atoms were absorbed from Wijs solution in 24 hours.

The behaviour of cinnamic acid, the Hübl value of which was determined by Lewkowitsch [1913], is in accordance with Bauer's rule [1904] for the addition of bromine to unsaturated substances, *i.e.* the capability of the ethylene linkage to add on bromine is diminished by the attachment of one of the negative groups COOH, COOR, C₆H₅ or Br to both carbon atoms. The C₆H₅ and CH₂OH groups (cinnamyl alcohol) or the CH₃ and COOH groups (crotonic acid) when attached to the two ethylene carbon atoms appear to be sufficient almost entirely to prevent formation of the iodochloride, although they do not prevent bromination.

The iodine values of the unsaturated ketones were determined by Ingle [1904]; his numbers are given below.

Substance	No. I atoms absorbed by 1 mol. substance	
	Hübl	Wijs
Benzylidene-acetone (Ingle)	2	—
C ₆ H ₅ CH : CH.CO.CH ₃		
Benzylidene-acetophenone (Ingle)	0.9	1.16
C ₆ H ₅ CH : CH.CO.C ₆ H ₅		4.2
Dibenzylidene-acetone (Ingle)	2.0	—
C ₆ H ₅ CH : CH.CO.CH : CH.C ₆ H ₅		
Benzal-cinnamylidene-acetone (Ingle)	4.0	—
C ₆ H ₅ CH : CH.CH : CH.CO.CH : CH.C ₆ H ₅		
Dicinnamylidene-acetone (Ingle)	6.0	—
C ₆ H ₅ CH : CH.CH : CH.CO.CH : CH.CH : CH.C ₆ H ₅		

Just as in the case of the unsaturated conjugated aldehydes, the values found are always below those required by the number of double bonds present in the molecule.

As we had at our disposal specimens of tetraphenylallene, tetraphenylethylene and diphenylbutadiene, we thought it would be of some interest to examine the effect of the conjugated ethenoid linkages and to compare the action of these unsaturated hydrocarbons when treated with Wijs reagent.

Substance and formula	No. I atoms absorbed by 1 mol. substance (Wijs)	Observer
Styrolene $\text{C}_6\text{H}_5\text{CH}:\text{CH}_2$	2	Ingle
Stilbene $\text{C}_6\text{H}_5\cdot\text{HC}:\text{CH}\cdot\text{C}_6\text{H}_5$	2	"
Tetraphenylethylene $(\text{C}_6\text{H}_5)_2\text{C}:\text{C}:(\text{C}_6\text{H}_5)_2$	0	MacLean and Thomas
Tetraphenylallene $(\text{C}_6\text{H}_5)_3\text{C}:\text{C}:\text{C}:(\text{C}_6\text{H}_5)_2$	2	"
Diphenylbutadiene $\text{C}_6\text{H}_5\cdot\text{CH}:\text{CH}:\text{CH}:\text{CH}\cdot\text{C}_6\text{H}_5$	4	"
Diphenyloctatetrene $\text{C}_6\text{H}_5\text{CH}:\text{CH}:\text{CH}:\text{CH}:\text{CH}:\text{CH}:\text{CH}:\text{CH}:\text{C}_6\text{H}_5$	6	"

It is perhaps worthy of note that the allene structure even when each allene terminal carbon atom is attached to two phenyl groups adds on two halogen atoms.

(2) *Hydrocarbons containing the phenanthrene nucleus.*

Phenanthrene and its 1-methyl-7-isopropyl derivative, retene, show a marked difference between the action of the two iodine reagents, the Wijs values being considerably higher than the Hübl numbers in both cases. The Wijs value was extremely variable, increasing with the excess of iodine used and with rise of temperature. Numbers lying between 26 and 101 were obtained. Anthracene differs from phenanthrene in that it absorbs halogen from both the Hübl and Wijs solutions to an almost equal extent. The only reduced phenanthrene derivatives we had an opportunity of studying were abietic and dihydroabietic acids. Although the presence of the carboxyl group introduces a new factor, the difference in behaviour of the reduced and unreduced acids suggests that the partially reduced phenanthrene nucleus exercises an important influence.

(3) *The sterols.*

The Hübl iodine values of the sterols cholesterol, brassicasterol and ergosterol correspond approximately with the absorption required by the presence of one, two or three double bonds respectively in their molecules. This is in agreement with what is known of the constitution of these substances. The Wijs values vary within wide limits but may be from two to three times as much as those obtained by Hübl's method.

Factors influencing the iodine values of the sterols.

Our experience shows that when the sterols are treated with iodine reagents, the iodine value obtained is largely influenced by (i) the time during which the sterol is in contact with the iodine solution; (ii) the relative concentration of iodine and sterol and (iii) the temperature of the reaction. The Hübl values are much less affected by these conditions than are the Wijs values and the former are always considerably lower than the latter.

(i) Influence of the time of reaction.

The iodine value determined after leaving 0.1 g. of cholesterol in contact with 15 cc. of the reagent for varying periods of time is shown in the following table:

No. of hours	Hübl	Wijs
6	58.65	141.6
18	70.2	152.7
24	73.2	153.7
48	71.8	165.4

(Theory for one double bond in cholesterol $C_{27}H_{46}O \cdot H_2O$ requires iodine value 62.8.)

After 18 hours' contact, the Hübl value is practically stationary; the Wijs number increases somewhat on further standing.

(ii) Relative concentration of iodine and sterol.

Quantities, each of 0.1 g. of cholesterol, were left for 24 hours with varying amounts of iodine solution; the results were as follows:

Weight of cholesterol	No. of cc. of iodine reagent	Hübl iodine value	Wijs iodine value
0.1 g.	10	71.45	103.6
"	15	72.8	132.9
"	20	73.2	164.3
"	25	72.1	172.6
"	30	73.3	180.5

(Theory for one double bond 62.8.)

The absorption from Wijs solution is markedly influenced therefore by increasing the relative proportion of the reagent; with Hübl's solution, on the other hand, this factor is almost without effect.

(iii) Influence of temperature.

When the iodine value of cholesterol was determined by the Wijs method, the same proportions of sterol and Wijs solution being left in contact for 24 hours, there were still wide variations in the values obtained, and we conclude that the day's temperature must exert a marked influence. The effect of increasing the temperature is shown in the following table:

Temperature			Hübl iodine value	Wijs iodine value
(Cold room)	...	0°	72.8	106.2
(Antechamber to cold room)	...	10°-13°	74.7	115
(Laboratory)	...	Between 15° and 20°	74.6	128
(Incubator)	...	26°	76.5	144
(Hot room)	...	36°	74.5	174

0.1 g. of cholesterol was treated with 15 cc. of the iodine solution for 24 hours.

At 36° the Hübl control showed a considerable diminution, the amount of available halogen in it having decreased. The increase with rise of temperature is again very much larger in the case of the Wijs than of the Hübl solution.

With Hübl's solution the values obtained were always slightly greater than those required by the presence of one double bond in the molecule. The highest value obtained corresponded to the absorption of $1\frac{1}{2}$ molecules of halogen. As long as the sterol and iodine solution were left together for 24 hours, the result was very little influenced by the relative concentrations of iodine reagent and sterol. It was somewhat more influenced by a rise in the external temperature.

With the Wijs reagent on the other hand, the iodine values are markedly affected both by an increase in the external temperature and by an increase in the relative amount of the Wijs reagent. The Wijs numbers are from two to three times as great as the Hübl values. The numbers obtained in an ordinary laboratory determination vary markedly, being affected both by the temperature of the day and the proportion of sterol to available halogen in the Wijs solution.

A specimen of dihydrocholesterol, originally obtained by Willstätter and Mayer [1908] by reducing cholesterol with hydrogen in the presence of platinum black, melted at 139°–140° (uncorrected) and showed a specific rotation of + 21°. From neither iodine reagent was an appreciable amount of iodine absorbed. A specimen of coprosterol, for which our thanks are due to Mr J. A. Gardner, showed a low iodine value of the same order with both reagents. As the number obtained corresponded to the absorption of less than one atom of iodine, it may have been due to some small amount of impurity in the specimen, or it may be conditioned by the substance itself.

The difference in the behaviour of the two reagents when reacting with the unreduced sterols is therefore associated with the presence of the double bond in the reduced nucleus.

It is difficult to explain the very high values obtained with the Wijs solution in the cases enumerated otherwise than by assuming that a large amount of substitution takes place. The use of iodine monochloride in glacial acetic solution as the halogenating agent, and the presence in the structure to be attacked of a reduced nucleus of condensed rings in which one double bond is present, appear specially favourable conditions.

(4) *The resins—abietic acid.*

Abnormal Wijs iodine values appear to be characteristic of the resin acids. The chief constituent of American colophony, known as abietic acid, has a much higher iodine value when treated with Wijs' than with Hübl's reagent. It is described as crystallising in colourless triangular plates, which are easily oxidised by the action of air, much more readily if kept in solution. Since

there are scarcely two observers who agree in the values they assign to its rotation and melting point, it is believed to be a mixture of two or more isomorphous acids. Rotations from $+66^\circ$ [Henry, 1901] to -66° [Mach, 1894] have been assigned to it, whilst the melting points ascribed vary from 120° [Flückiger, 1887] to 198° [Klason and Köhler, 1906].

Pimaric acid, isolated from French resin (galipot), melting at 211° – 212° and having a specific rotation of $+75.5^\circ$, is considered by Easterfield and Bagley [1904] as the next homologue of abietic acid.

The resinic acids cannot yet be said to have been satisfactorily separated into individual substances of undoubted identity. The difficulty is increased since resins of different origins apparently vary in their constituents. Klason and Köhler [1906] succeeded in isolating from certain Scandinavian resins two acids, one melting at 168° – 173° , $[\alpha_D] = +52^\circ$, and one melting at 198° , $[\alpha_D] = -60^\circ$. These acids closely resemble each other in chemical properties and it seems probable that what has been described as abietic acid is a mixture of these or of closely related acids.

We tried various methods described for the preparation of abietic acid and found most satisfactory the repeated distillation of the resin at 20 mm. pressure, with subsequent recrystallisations from methyl and ethyl alcohols [Easterfield and Bagley, 1904; Levy, 1905]. We found that if the recrystallisations are carried out at 0° , it is easier to obtain a specimen free from a yellow tinge. We did not, however, succeed in raising our melting point to 182° as described by Levy. Working in this way with two different specimens of resin, both sold to us as American colophony, we obtained from one an acid which softened at 155° and melted at 162° , $[\alpha_D] = -73.5^\circ$, and from another an acid melting at 166° after softening at 154° , $[\alpha_D] = +37^\circ$. On examination, the original resins were found to be respectively laevo- and dextro-rotatory. The dextro-acid seemed to show less tendency to become coloured; solutions in alcohol, left exposed to the light for six weeks in stoppered bottles, remained colourless.

The laevo-specimen of the acid was reduced with platinum black and hydrogen in ether solution and a compound obtained which melted after several recrystallisations at 165° – 167° , and had $[\alpha_D] = -88.1^\circ$. This substance is probably similar to that obtained by Johanssohn [1917] by reducing under similar conditions a specimen of abietic acid which melted from 168° – 173° and had $[\alpha_D] = -28.6^\circ$. His reduced acid softened at 156° and melted from 173° – 178° , the rotation not being given. In our case the specific rotation of the acid was raised by reduction from -73.5° to -88° .

Iodine values.

Fahrion [1901], Vesterberg [1907] and Johanssohn [1917] examined the iodine values of these acids using the Hübl-Waller method. Johanssohn found considerable variations according to the concentration and time of contact

with the iodine solution but made the curious observation that the iodine value of the reduced acid was practically identical with that of the original acid and showed this was also true for pimelic and dihydropimelic acid.

If abietic acid and its dihydro-derivative are examined by the Hübl and Wijs methods almost identical values are obtained with the latter reagent but the Hübl solution differentiates between them, the value for the dihydro-acid having been reduced by the amount required by one double bond.

	Hübl-Waller iodine value	No. I atoms added per mol. substance	Hübl iodine value	No. I atoms added per mol. substance	Wijs's iodine value	No. I atoms added per mol. substance
Abietic acid ...	173.9 ¹ 164 ²	4	162	4	239	6
Dihydro-abietic acid	166 ²	4	75	2	232	6
Cholesterol ...	—	—	72	2.3	158	5

¹ Fahrion.

² Johanssohn.

If the difference between the Hübl and Wijs values represents the absorption due to substitution, in dihydro-abietic acid twice as much substitution takes place as in abietic acid itself. In dihydro-abietic acid as in cholesterol the presence of a condensed ring nucleus containing one double bond seems to exert a considerable influence. In dihydro-abietic acid the nucleus has almost certainly a reduced phenanthrene structure.

The main points on which a formula for abietic acid [cp. Levy, 1913] may be based are:

(1) Analysis and neutralisation values agree best with the formula $C_{20}H_{30}O_2$ [Levy, 1905].

(2) The COOH group present is not esterified by the action of alcohol and HCl; therefore there are probably two substituents in the ortho-position to the carboxyl groups [Easterfield and Bagley, 1904].

(3) By oxidation with nitric acid, hexahydrophthalic acid has been obtained, pointing to the presence of a terminal hexahydrobenzene ring without substituents [Levy, 1905].

(4) On distilling abietic acid with sulphur, retene (1-methyl-7-isopropyl-phenanthrene) is obtained [Vesterberg, 1903], so that a phenanthrene nucleus is probably present in abietic acid, or else a group which will readily pass into phenanthrene [Levy, 1913].

If a terminal hexahydrobenzene nucleus be present in the abietic acid molecule, the wandering of a methyl group during the distillation must be assumed.

(5) An isopropyl group is present, since isobutyric acid was obtained from the products of oxidation with permanganate [Levy, 1906].

(6) Four halogen atoms are absorbed from Hübl's solution, agreeing with presence of two double bonds, neither being in the α , β -position to the COOH group.

(7) Reduction at ordinary temperature in ethereal solution with hydrogen in the presence of platinum black leads to the addition of two hydrogen atoms [Johannsohn, 1917]; the dihydro-abietic acid thus formed contains one double bond in the molecule, since it still reacts with both iodine solutions.

Neither the position of the double bonds nor that of the alkyl substituents in abietic acid is definitely established, but the above facts are represented by some such formula as the following:

The most recent formula ascribed by Windaus [1920] to cholesterol is added for the purpose of comparison.

CONCLUSIONS.

1. When iodine values are determined by both the Wijs and the Hübl methods the Hübl iodine values obtained are much higher than the Wijs in the case of phenol and to a less extent of α -naphthol. Since the Wijs reagent acts as an enolising agent, a possible explanation is that a change to keto-dihydrobenzene precedes the addition of halogen and that this change is retarded by the Wijs reagent [cp. Ingle, 1904].

2. The Wijs iodine numbers are much higher than the Hübl values in the case of four sets of compounds amongst those examined: (a) fatty carbonyl compounds, (b) the phenanthrene hydrocarbons, (c) sterols and (d) the resins.

3. It is suggested that, in accordance with Lapworth's work, the carbonyl compounds are enolised by the acid of the Wijs reagent and that the addition of halogen then takes place. This enolising action is brought about only very slightly by the neutral Hübl reagent.

In carbonyl groups with conjugated ethenoid groups, where enolisation does not so readily occur, the Hübl values are equal to or greater than those given by the Wijs reagent.

4. Anthracene absorbs from 2 to 4 halogen atoms and does not differentiate between the Hübl and Wijs reagents. Phenanthrene absorbs up to 1.31 atoms

from the Wijs solution but is almost without action on the Hübl. The Wijs value depends largely on temperature and the relative iodine concentration.

5. The conversion of anthracene and phenanthrene to the corresponding quinones completely prevents halogenation by the iodine reagents.

6. Abietic acid reacts with Hübl and Wijs solutions, the iodine values obtained corresponding to the absorption of approximately 4 and 6 atoms of iodine respectively. The Hübl value indicates that two double bonds are present in the abietic acid molecule, which is in accordance with what we know of its constitution.

7. Addition of two hydrogen atoms to the abietic acid molecule leaves the Wijs value unchanged but reduces the Hübl value to that indicating the presence of one double bond in the dihydro-abietic acid formed.

8. The Hübl values of the sterols are variable but indicate approximately the number of double bonds believed to be present in the molecule. The Wijs numbers are very much greater and probably indicate that a considerable amount of substitution has taken place. The reduced sterols show small iodine values whether examined by the Hübl or Wijs reagent.

9. In the sterols and resins the presence of a condensed ring nucleus containing one or two double bonds influences the amount of substitution produced by the Wijs reagent. The actual number of double bonds present is approximately indicated if the iodine value be determined by Hübl's solution.

10. In any fat containing appreciable quantities of sterol, the Hübl iodine value more accurately represents the degree of unsaturation of the mixture than does the Wijs value. The Wijs values of many fats are appreciably higher than the Hübl numbers [Wijs, 1898]. This difference is significant and indicates the presence of sterol in the fat.

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