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Factors Giving Rise to the Abnormal Iodine Absorption Number of Cholesterol

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Introduction

Cholesterol has been found to give abnormal iodine numbers when a modified Hanus procedure was used.[1] This fact was also observed by Rosenmund and Kuhnhenn[2] and by Dam,[3] who, in addition, showed that the abnormal values were a function of the quantity of excess reagent and the duration of the reaction. They used the Wijs, Hanus, Hüble, Waller and the pyridine sulfate dibromide methods. The author has found, however, that in using the modified Hanus procedure at 1°, the theoretical iodine number of 65.7 can be obtained consistently. This is in marked contrast to the higher values of 72 and above obtained at 25°.

The literature, to the author's knowledge, affords no adequate explanation for these discrepant values. Recently, Buckwalter and Wagner,[4] working with other substances, observed a "thermal cleavage of halogen acid" from substances undergoing halogenation. It was accompanied by an excessive consumption of halogen, which gave rise to abnormal iodine numbers. These actions were diminished at low temperatures. Now substitution and oxidation reactions generally have larger temperature coefficients than do addition reactions. Therefore, it seemed possible that substitution or oxidation or a combination of both was responsible for the iodine values in excess of 65.7. The work reported here was undertaken to determine the character of the side reactions, of the products formed and of the groups involved.

Materials

Cholesterol (Eastman Kodak Co.) was used in the preparation of materials for this work. It was purified by the methods described by Koch, Koch and Kraus-Ragins.[5] The dibromocholesterol was reduced to cholesterol by means of zinc dust and acetic acid,[6] m. p. 147.5°.

Ergosterol-free cholesterol was supplied by Mead-Johnson and Company.[7]

Cholesteryl acetate was prepared and purified as described by Anderson,[8] m. p. 114.1°.

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(1) While at St. Louis University School of Medicine, the author and C. N. Jordan modified the Hanus procedure for use on 15–25 mg. of material in determining the iodine number of ovarian preparations.

(2) Rosenmund and Kuhnhenn, *Z. Unters. Nahr. Genuss.*, **46**, 154 (1923).

(3) Dam, *Biochem. Z.*, **152**, 101 (1924).

(4) Buckwalter and Wagner, THIS JOURNAL, **52**, 5241 (1930).

(5) Koch, Koch and Kraus-Ragins, *J. Biol. Chem.*, **85**, 141 (1929–1930).

(6) Hess and Anderson, *ibid.*, **74**, 651 (1927).

(7) The author wishes to express his gratitude to Mead-Johnson and Co.

(8) Anderson, *J. Biol. Chem.*, **71**, 407 (1926–1927).

Cholesteryl benzoate was prepared by a procedure described by Heyl and Swoap[9] for the preparation of ergosteryl benzoate. It was purified in the manner described by Shriner and Ko,[10] m. p. 148°.

Triphenylmethyl cholesteryl ether was prepared as described by Josephson,[11] m. p. 139.0°.

Dihydrocholesterol (**β-Cholestanol**).[12]—One preparation was made in this Laboratory by the method described by Anderson and Nabenhauer[13] for the reduction of sitosterol, excepting that the catalyst was the platinum oxide described by Shriner, Adams and Voorhees.[14] Purification was effected according to Anderson, Nabenhauer and Shriner,[15] m. p. 142.0°.

Cholestenone was prepared by the CuO method of Diels and Abderhalden,[16] m. p. 81.0°.

Dehydrocholestanone-7-ol-4 was prepared from nitrocholesteryl nitrate[17] by a method described by Windaus,[18] m. p. 141–142°.

Dibromocholesterol was prepared by bromination of cholesterol in a mixture of ether and glacial acetic acid (4:1),[8] m. p. 119°.

Dibromocholesteryl acetate and dibromocholesteryl benzoate were made from cholesteryl acetate and benzoate, respectively, in the same manner as dibromocholesterol; respective melting points 116 and 132°.

Methods

The reagents used were the Hanus solution diluted to half strength, a solution of iodine monobromide in carbon tetrachloride equivalent to the diluted Hanus reagent, and a pyridine sulfate dibromide solution of the same halogen content as those above.[3]

The materials to be studied were dissolved in chloroform, carbon tetrachloride or glacial acetic acid, as the particular experiment demanded, so that 2 cc. of solution contained 15 mg. of cholesterol or its equivalent in the form of a derivative. To each 2 cc. of these solutions, 4 cc. of halogen reagent was added. After stated intervals of time, 6-cc. portions of the reaction mixtures were withdrawn for appropriate analyses.

Total halogen consumed was determined by titration of the remaining halogen with 0.07 *N* sodium thiosulfate in the presence of 5 cc. of 0.05 *N* potassium iodide. The sodium thiosulfate was standardized at frequent intervals and the necessary blanks accompanied each determination.

Total inorganic halogen was determined by running the 6-cc. aliquot portion into 5 cc. of sodium arsenite of appropriate concentration. When the two solutions had been mixed and the halogen reduced, water was added to make the aqueous volume up to 35 cc. The mixture was stirred and then allowed to separate into two layers. The water was drawn off into a 35 × 200-mm. test-tube and saved. The non-aqueous layer was washed three times with 10-cc. portions of water. The halogen in these combined water solutions was determined by the Volhard procedure. Blanks were run in the same manner.

Organically bound halogen is represented by the difference between the total inorganic halogen of a blank and that of an experimental run. For a time the organic

(9) Heyl and Swoap, THIS JOURNAL, **52**, 3688 (1930).

(10) Shriner and Ko, *J. Biol. Chem.*, **80**, 1 (1928).

(11) Josephson, *Ann.*, **493**, 174 (1932).

(12) The writer is indebted to Dr. R. L. Shriner of the University of Illinois, who made another preparation of dihydrocholesterol used in this work.

(13) Anderson and Nabenhauer, THIS JOURNAL, **46**, 1953 (1924).

(14) Shriner, Adams and Voorhees, *Org. Syn.*, **8**, 92 (1928).

(15) Anderson, Nabenhauer and Shriner, *J. Biol. Chem.*, **71**, 389 (1926–1927).

(16) Diels and Abderhalden, *Ber.*, **37**, 3099 (1904).

(17) Preis and Raymann, *ibid.*, **12**, 225 (1879).

(18) Windaus, *ibid.*, **36**, 3754 (1903).

halogen was determined on the contents of the organic solvent layer after removal of the inorganic halogen, but this was abandoned as soon as it had been repeatedly shown to give results that checked with those of the former method.

Halogen acid produced during a reaction is represented by the difference between the total halogen consumed and the organic halogen. In neutral solvents, the figures determined in this way checked with those obtained by means of potassium iodate and titration with sodium thiosulfate.[8]

The organically bound, the acid and the total consumed halogen were expressed and plotted as atoms or equivalents per mole of cholesterol or its equivalent. This rendered comparison of the results more understandable when different derivatives of cholesterol were studied.

After recovery of the organic products of the reactions, by careful evaporation of the organic solvent on a water-bath and with the aid of a stream of compressed air, ketones were isolated by means of 2,4-dinitrophenylhydrazine in the manner described by Allen,[19] excepting that no water was added to induce precipitation. After a few minutes of standing an amorphous precipitate formed which was filtered and dried by suction. This was then almost dissolved in a minimum of hot normal butyl alcohol. Solution was completed by the addition of a few drops of benzene. When this solution cooled slowly, crystals formed. Recrystallization from butyl alcohol and benzene was effected several times until well-formed sharp-melting crystals were obtained.

The halogen in isolated derivatives was determined by boiling a weighed quantity of the substance with a mixture of glacial acetic acid, nitric acid and a known quantity of silver nitrate for one and one-half hours. The excess silver nitrate was titrated with ammonium thiocyanate. The method was tested on pure samples of dibromocholesterol, dibromocholesteryl acetate and dibromocholesteryl benzoate.

The nitrogen content of derivatives was determined in the following manner. A weighed sample was dissolved in glacial acetic acid which was then diluted with a little alcohol and water. About 0.5 g. of zinc dust was added and the mixture boiled for two hours. Then 2 cc. of concd. sulfuric acid was added and the mixture evaporated until sulfur trioxide fumes filled the tube. At this time, a few drops of Superoxol (30% H_2O_2) were added and digestion was continued until the solution was colorless. After the digest had cooled, it was made alkaline and the ammonia was aerated into an excess of standard acid. The excess acid was titrated with a standard alkali and the nitrogen content of the sample was calculated. The theoretical results were obtained for the nitrogen content of pure 2,4-dinitrophenylhydrazine. Inasmuch as this substance was the only source of nitrogen in our samples, the method was considered adequate.

Results and Discussion

1. As shown by Fig. 1, at 25° and after ninety minutes of reaction time, in carbon tetrachloride, cholesterol yielded, with the diluted Hanus reagent, iodine values ranging only from 112 to 117% of the theoretical, whereas, in chloroform, it gave values ranging from 137 to 175% of 65.7. In chloroform, purified by treatment with calcium oxide, sodium bisulfite, and phosphorus pentoxide and fractional distillation, the values were still 50% too high. Thus these results indicate, as do those of Böeseken and Gelber,[20] that chloroform is not a good solvent for unsaturation value determinations and that, as Buckwalter and Wagner[4] also contend, carbon tetrachloride, not chloroform, should be used.

(19) Allen, THIS JOURNAL, **52**, 2955 (1930).

(20) Böeseken and Gelber, *Rec. trav. chim.*, **46**, 158 (1927).

2. Since using carbon tetrachloride as the solvent did not lead to the theoretical values and since the consumption of halogen did not cease, but continued over a long period of time (Fig. 2) when the modified Hanus procedure was used, it was thought that the pyridine sulfate dibromide method might yield better results. This method (1, 2) is said to give the theoretical values for cholesterol, but, as may be seen in Fig. 2, it is subject to the same errors as the Hanus procedure. While the consumption of halogen is more nearly the theoretical, that organically bound is much too low.

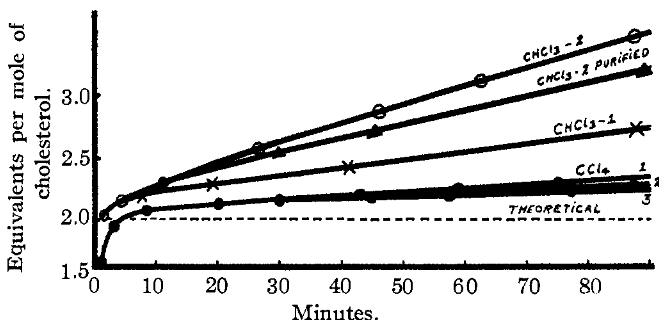

Fig. 1.—Halogen absorbed by cholesterol *vs.* time of reaction: solvents, $CHCl_3$ and CCl_4; temp., 25°.

Determinations of iodine numbers on ultra pure cholesterol indicate that the anomalous figures are not caused by the presence of impurities (at least not by common impurities).

Inasmuch as some doubt[21] still exists as to the exact structure of cholesterol and since the formula is still given as $C_{27}H_{43}OH$ or $C_{27}H_{45}OH$, it was thought that there might be a second refractory and not easily detected double bond in the molecule. However, since the iodine value for cholesterol did not reach a limiting value (Fig. 2) representing a union of four or more whole atoms of halogen per mole of cholesterol even though the reaction was allowed to proceed for as long as twenty-four and forty-eight hours, there appeared to be no second double bond in the molecule.

3. Since the relations between the total halogen consumed, the halogen acid produced and the halogen organically bound during a reaction of cholesterol with a halogenating reagent would indicate the nature of the reactions occurring, *if the double bonds were fully saturated*, these determinations were made on cholesterol and its derivatives using modified Hanus reagents and pyridine sulfate dibromide in the hope that oxidation or substitution, together with addition, might be indicated. However, Figs. 2 and 3 show that the primary condition was not met, for the organic halogen was 80% of the theoretical, or 1.6 atoms of halogen per mole of sterol used.

(21) Steinle and Kahlenberg, *J. Biol. Chem.*, **67**, 461 (1926).

The results of Fig. 2, though somewhat disappointing, did raise an interesting question. Why was the bound halogen less than the theoretical even though the observed iodine values were much too high? In Fig. 3 it may be seen that the dibromo derivatives of cholesterol lose halogen when exposed to glacial acetic acid alone or containing iodine monobromide. The loss of halogen is greater, however, when the iodine monobromide is present and is accompanied by a marked consumption of that reagent. The bound halogen and the consumed halogen of these experiments approximated the organic halogen and the consumed halogen in excess of two atoms per mole of sterol when the non-halogenated cholesterol or derivatives were the starting materials.

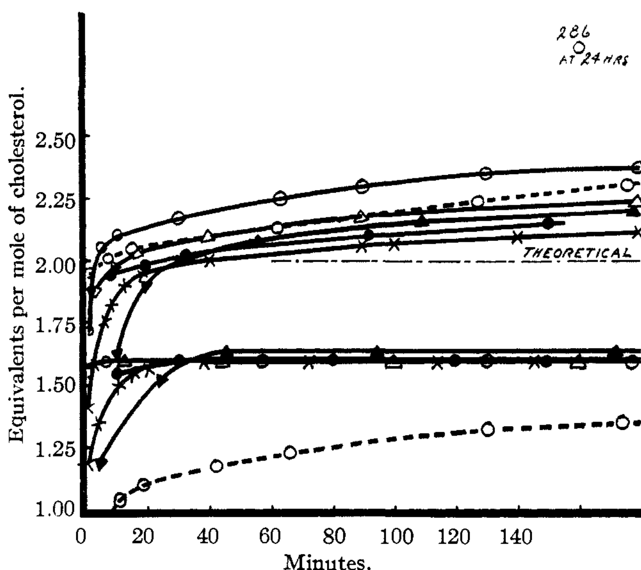

Fig. 2.—Total halogen consumed and organic halogen *vs.* time of reaction, temp., 25°: upper group of curves, total halogen consumed; lower group, organically bound halogen; —, modified Hanus procedure; – –, pyridine sulfate dibromide method; ○, purified commercial cholesterol; △, ergosterol-free cholesterol; ●, triphenylmethyl cholesteryl ether; ▲, cholesteryl benzoate; ×, cholesteryl acetate.

The loss of halogen from dibromocholesterol and its derivatives together with the less than theoretical bound halogen in our reaction mixtures might have been the result of the "reversible addition" of halogen described by Böeseken and Gelber.[20] These workers state that the presence of dissociating solvents, among which is included glacial acetic acid, and the presence of potassium iodide favor this action. However, when this possible cause was checked by allowing dibromocholesterol to stand in

contact with glacial acetic acid, with glacial acetic acid and a moderate concentration of potassium iodide, with glacial acetic acid and a relatively enormous concentration of potassium iodide, for periods of one, two, three, six and twelve hours, not more than 0.04–0.08 of 1 per cent. of the halogen of dibromocholesterol was liberated in a form that would give the starch iodide test. However, since in our usual procedure of determining combined and ionic halogen, sodium arsenite was present, it was conceivable that this substance might reduce any atomic halogen as fast as it was

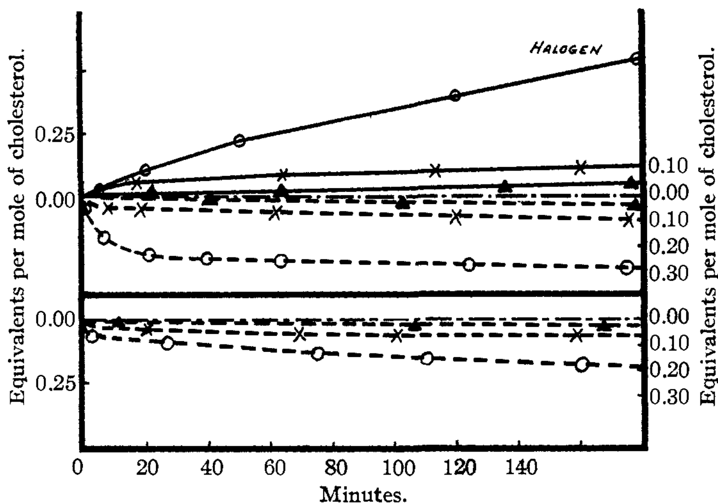

Fig. 3.—*Upper section.* Halogen consumed, and decrease in organic halogen *vs.* duration of contact with IBr in CCl_4 + CH_3COOH, temp., 25°. *Lower section.* Decrease in organic halogen *vs.* duration of contact with CCl_4 + CH_3COOH, temp., 25°: — halogen consumed; - - - decrease in organic halogen; ○, dibromocholesterol; ×, dibromocholesteryl acetate; ▲, dibromocholesteryl benzoate.

liberated from the sterol and thus continually upset any equilibrium and thereby increase the loss of halogen from the organic substance. If such were the facts, repeated applications of the regular procedure to the reaction products should have resulted in steadily decreasing values for the organic halogen. Moreover, if the procedure was applied to dibromocholesterol it should cause a loss of halogen from dibromocholesterol. However, neither repeated applications of the regular procedure nor marked variations in the quantities of the respective reagents caused more than a maximum change of 2% in the determined organic halogen. Nor was there any loss of halogen from dibromocholesterol treated in the same way. Therefore, it is indicated that the halogen which splits out of the halogenated sterol comes out in the form of halogen acid and that it does so during the main reaction period and not during subsequent analytical procedures.

Nor did it appear that the loss of halogen acid was due to the "thermal cleavage" of Buckwalter and Wagner,[4] because the organic halogen at 0° and at 25° was practically the same (Table I). It was evident, however, that temperature influenced the consumption of halogen, it being much greater at the higher temperature, especially when cholesterol was under examination.

It is plain that the solvent plays a prominent part in the cleavage of halogen acid from halogenated cholesterol. As shown in Table II, as the proportion of acetic acid present increases, the loss of halogen acid increases and the organic halogen decreases. Moreover, the presence of pyridine still further diminishes the organic halogen. In the case of pyridine, it was found that, by varying the ratio of the pyridine to the sulfuric acid present in the pyridine sulfate dibromide solution, the loss of halogen acid varied in the same direction. By reference to the work of Hall and Conant,[22] it was seen that the neutralization of pyridine by sulfuric acid in glacial acetic acid was reversible and had an equilibrium constant, approximately 3.74×10^{-4}. The free pyridine (calculated using this figure) in these reaction mixtures was sufficient to neutralize the increased quantities of halogen acid and thus could increase its tendency to split out.

Since the complete loss of halogen acid from dihalocholesterol could

TABLE I

THE EFFECT OF TEMPERATURE UPON THE TOTAL HALOGEN CONSUMED, ORGANIC HALOGEN, AND HALOGEN ACID PRODUCED

Substance		Cholesterol		Cholesteryl acetate	
Temperature, °C.		25	0	25	0
Division or class of halogen	Total consumed	2.30[a]	2.05	2.06	1.94
	Organic	**1.60**	**1.64**	**1.59**	**1.58**
	Acid produced	0.70	0.41	0.47	0.36

[a] The figures represent equivalents of halogen per mole of cholesterol or derivative. The reagent was IBr in glacial acetic acid and the solvent was carbon tetrachloride. The duration of the reaction was ninety minutes.

TABLE II

THE EFFECT OF THE COMPOSITION OF THE SOLVENT UPON TOTAL HALOGEN CONSUMED, ORGANIC HALOGEN, AND HALOGEN ACID PRODUCED

Composition of solvent		Pyridine HAc CCl_4	HAc	HAc + CCl_4	CCl_4
Division or class of halogen	Total consumed	2.18[a]	2.34	2.30	2.73
	Organic	**1.28**	**1.49**	**1.60**	**2.36**
	Acid produced	0.90	0.85	0.70	0.37

[a] The time of reaction was ninety minutes. The figures represent equivalents per mole of cholesterol.

(22) Hall and Conant, THIS JOURNAL, **49**, 3047 (1927).

result in the formation of either conjugated double bonds or a triple bond, these two groups were looked for in our products. Maleic anhydride[23] gave no indications of the former but there were other indications of the latter (discussed later).

4. In Table II, it will be seen that, when carbon tetrachloride was the solvent for both the sterol and the IBr, and the temperature was 25°, not only was there no loss of halogen acid during the reaction but there was a union of halogen with the sterol greater than that required to saturate one double bond. Table III shows that the data indicated that substitution and addition had taken place. It is therefore possible that substitution is one of the side reactions when the usual Hanus or pyridine sulfate dibromide procedure is used but is masked by other side reactions.

TABLE III

RELATIONS BETWEEN TOTAL CONSUMED, ORGANIC AND ACID HALOGEN WHEN CARBON TETRACHLORIDE IS THE SOLVENT

Temp., 25°

Time of reaction, min.	3	6	10	30	60	90
Total	2.00[a]	2.04	2.09	2.32	2.56	2.73
Organic	2.00	2.02	2.05	2.15	2.27	2.36
Acid	0.00	0.02	0.04	0.17	0.29	0.37
Org. hal. − 2	.00	.02	.05	.15	.27	.36
Ratio hal. acid/O. hal. − 2	....	1.0	.8	1.1	1.1	1.0

Temp., 0°

Time of reaction, min.	3	6	10	30	60	90
Total	1.93[a]	1.99	2.00	2.00	2.00	2.01
Organic	1.93	1.99	2.00	2.00	2.00	2.01
Acid	0.00	0.00	0.00	0.00	0.00	0.00
Org. hal. − 2	− .07	− .01	.00	.00	.00	+ .01

[a] The figures in this table represent equivalents of halogen per mole of cholesterol. They are to within −0.02 equivalents per mole. Time is in minutes from the time the reagents were mixed.

At 0°, however, with carbon tetrachloride as the sole solvent, the total halogen consumed and the organically bound halogen were equal and equivalent to 2 g. atoms of halogen per mole of sterol. It would seem, therefore, that iodine numbers of lipid fractions containing sterol should be determined at 0° with carbon tetrachloride as solvent for both reagent and sample.

5. Oxidation at the secondary alcohol group of cholesterol might well be the cause of the abnormal iodine values. However, fixation of that group by the acetyl, the benzoyl and the triphenylmethyl groups did not, at 25°, prevent an excessive consumption of halogen by those cholesterol derivatives. They did, however, consume less than did cholesterol (Fig. 2).

(23) Windaus and Luttringhaus, *Ber.*, **64**, 850 (1931).

These results, together with the fact that dihydrocholesterol (β-cholestanol), in which the alcohol group is free, did not react at all with IBr, would seem to indicate that the secondary alcohol group is not greatly involved in the production of the anomalous values of cholesterol. However, when the double bond is saturated, there is still a considerable consumption of halogen by cholesterol and but very little by the benzoate or the acetate (Fig. 3). This would lead one to believe that the alcohol group was involved.

Because the oxidation of cholesterol by the Hanus reagent could not be demonstrated by other means, the isolation of possible products of such action was attempted. Among them would be ketones. The action of 2,4-dinitrophenylhydrazine upon the products of the reaction of cholesterol, ergosterol-free cholesterol, and certain samples of dibromocholesterol with IBr or pyridine sulfate dibromide at 25° yielded a red precipitate, which upon recrystallization formed feathery needles melting at 231–232°. At this time attempted preparations of cholestenone (one of the possible ketones) were not overly successful, but the reaction mixtures, with dinitrophenylhydrazine, yielded a crystalline compound identical with that described above. They melted at the same temperature separately and when mixed. They contained an average of 10% nitrogen and no halogen. The calculated molecular weight (assuming four nitrogens to the molecule) was 560; that of the dinitrophenylhydrazone of cholestenone is 564. While the hydrazone of cholestenone was indicated, the fact that we had not been able to establish that oxidation had occurred at the alcohol group, and the fact that the methods described for the preparation of cholestenone do not absolutely protect the double bond, left open the possibility of oxidation at that point.

Oxidation at the double bond could result from the cleavage of two molecules of halogen acid from dihalocholesterol (this cleavage has been shown to occur) and subsequent reaction with water. Sufficient water for this is present in the glacial acetic acid of our reaction mixtures. The product of these changes would contain a carbonyl group on either of the carbon atoms of the old double bond. Cholesterol treated with halogen under conditions favorable to the cleavage of halogen acid (a high concn. of acetic acid or the presence of pyridine) yielded, with dinitrophenylhydrazine, a mixture of a red and a yellow derivative. Upon fractional crystallization, these were separated. The yellow needle-like clusters melted at 257°, while the red crystals melted at 231–232° and were identical with the previously described red crystals. Crystals like the yellow ones were obtained after treatment of dibromocholesterol with pyridine, acetic acid, silver nitrate and then with dinitrophenylhydrazine. The two derivatives could be the hydrazones of dehydrocholestanone-7-ol-4 and dehydrocholestanone-6-ol-4. No method for the preparation of the latter

could be found. The former was prepared. Cholestenone was also prepared and isolated in crystalline form. By means of a few microscopic measurements, the general crystalline form and melting points, mixed and separate, it was established that the 2,4-dinitrophenylhydrazone of dehydrocholestanone-7-ol-4 was identical with our yellow derivative while that of cholestenone was identical with the red compound.

In Table IV it will be seen that, at 25°, oxidation occurs at the alcohol group and the double bond (in presence of acetic acid or pyridine). At 0°, only the latter occurs. In carbon tetrachloride as the sole solvent, neither oxidation takes place.

TABLE IV

THE PRODUCTION OF KETONES FROM CHOLESTEROL AND ITS DERIVATIVES AND THE PHYSICAL PROPERTIES OF THE ISOLATED DINITROPHENYLHYDRAZONES

Material used	Procedure	Temp., °C.	Result[a]	Properties of dinitrophenylhydrazone Color[b]	M. p., °C.
Cholesterol (com. and pure)	IBr, CH_3COOH, CCl_4	25	+	RED, yellow	231–232
Cholesterol	IBr, CH_3COOH, CCl_4	0	?	Yellow	
Cholesterol	Pyridine sulfate dibromide		+	RED, yellow	231–232 257
Cholesterol	IBr, CCl_4	25	−		
Cholesterol	IBr, CCl_4	0	−		
Cholesterol	Br_2, ether, glacial HAc	25	+	RED	231
Cholesterol	Br_2, $KMnO_4$, H_2SO_4	25	+	RED	232
Cholesterol	Br_2, $KMnO_4$, H_2SO_4, HAc and Zn		+	RED	230
Cholesterol	Fused with CuO	260	+	RED	231
Cholesteryl acetate	IBr, CH_3COOH, CCl_4	25	?		
Cholesteryl acetate	IBr, CH_3COOH, CCl_4	0	−		
Cholesteryl benzoate	IBr, CH_3COOH, CCl_4	25	?		
Triphenylmethyl cholesteryl ether	IBr, CH_3COOH, CCl_4	25	?		
Dibromocholesterol	IBr, CH_3COOH, CCl_4	25	+	Red, yellow	230
Dibromocholesterol	CH_3COOH	25	?	Yellow	
Dibromocholesterol	CH_3COOH and pyridine	25	+	YELLOW	257
Dibromocholesterol	CH_3COOH and $AgNO_3$	25	+	YELLOW	257
Dibromocholesterol	CH_3COOH, no subsequent H_2O	25	+	YELLOW	257
Dibromocholesteryl acetate	IBr, CH_3COOH, CCl_4	25	?	Red, yellow	
Dibromocholesteryl acetate	CH_3COOH, CCl_4	25	?	Yellow	
Dibromocholesteryl benzoate	IBr, CH_3COOH, CCl_4	25	?	Yellow	
Dibromocholesteryl benzoate	CH_3COOH, CCl_4	25	?		
Dihydrocholesterol	IBr, CH_3COOH, CCl_4	25	No action		
Cholestenone				RED	231.5
Dehydrocholestanone-7-ol-4				YELLOW	257

[a] In the "result" column + indicates an isolation of ketone as the 2,4-dinitrophenylhydrazone; − indicates that no ketone was isolated; and ? indicates that only traces were formed. [b] The size of the letters in the "color" column indicates the relative amount of each hydrazone isolated.

The fact that the Lifschütz reaction[24] was given by the products of the reaction of cholesterol with the Hanus reagent and with pyridine sulfate dibromide, and that it was not given by cholestenone, dehydrocholestanone-7-ol-4, or any other of the known products of these reactions, leads us to believe that there must be other oxidative products formed in

(24) Lifschütz, *Ber.*, **41**, 252 (1908).

our reaction mixtures. The nature of oxycholesterol is very indefinite,[25] although Lifschütz[26] assigns it the formula $C_{27}H_{46}O_2$. Windaus[27] has isolated an oxycholesterol of the same formula, but its identity with that of Lifschütz has not been established.

6. Why did the dinitrophenylhydrazone of cholestenone, isolated from the products of the reaction of cholesterol with IBr, contain no halogen? According to Böeseken and Gelber,[20] the proximity of a double bond to a negative group, such as C=O, renders that bond resistant to halogenation. In Table V it will be seen that cholestenone treated with the Hanus reagent consumed, by the end of three hours, only 58% of the theoretical quantity of halogen and was actually combined with only 50% of that. It appears from the data that there had occurred a slow saturation of some of the molecules which was accompanied by a slower cleavage of halogen acid from those same molecules; 42% of the cholestenone was unaffected. The nature of products of the reactions was not ascertained but among them may have been oxycholestenone and/or cholestandione-7,4.

TABLE V

THE BEHAVIOR OF CHOLESTENONE WITH IODINE MONOBROMIDE IN CARBON TETRACHLORIDE AND GLACIAL ACETIC ACID AT 25°

Reaction time	40 minutes		90 minutes		180 minutes	
	Expressed in terms of					
Distribution between	Equiv. per mole	Per cent. of calcd.[a]	Equiv. per mole	Per cent. of calcd.	Equiv. per mole	Per cent. of calcd.
Total halogen	0.33	16.8	0.61	30.7	1.17	58.7
Organic halogen	0.28	14.1	0.45	22.4	0.58	28.9
Halogen acid	0.05		0.16		0.59	

[a] Calcd. is 2.00 equivalents of total halogen and organic halogen, respectively, and no halogen acid at all.

Summary

1. The main reaction when cholesterol is treated with iodine monobromide or pyridine sulfate dibromide in glacial acetic acid is addition at the double bond. The product is dihalocholesterol.

2. The dihalocholesterol loses halogen acid at 25° and 0° in glacial acetic acid. The presence of pyridine augments this loss. The result is the formation of dehydrocholestanone-7-ol-4, whose dinitrophenylhydrazone crystallizes in clusters of burnt-ocher colored needles that melt, with decomposition, at 257°.

3. Cholesterol is oxidized to cholestenone when allowed to react with iodine monobromide or pyridine sulfate dibromide in glacial acetic acid at 25°. Cholestenone forms a 2,4-dinitrophenylhydrazone, red in color, which crystallizes as feathery needles melting at 231–232°.

4. Cholesterol and its derivatives yield abnormally high iodine numbers

(25) Windaus, *Biochem. Handl.*, **3**, 272, note 18 (1911).

(26) Lifschütz and Grethe, *Ber.*, **47**, 1453 (1914).

(27) Westphalen and Windaus, *ibid.*, **48**, 1064 (1915).

at 25°, *but the organically bound halogen is less than the theoretical* due to the concurrent cleavage of halogen acid with the production of dehydrocholestanol which cannot add halogen and the formation of cholestenone which does not readily add halogen. Other substances of similar nature may be involved.

5. At 25°, cholesterol undergoes substitution and addition when carbon tetrachloride is the solvent for both the sterol and the halogen reagent.
6. At 0°, with carbon tetrachloride as the sole solvent, cholesterol undergoes addition only and the iodine numbers are the theoretical.
7. Besides dibromocholesterol, cholestenone, dehydrocholestanone-7-ol-4, and probably some dibromocholestenone, other products are formed by the action of the Hanus reagent upon cholesterol. The nature of these substances is not known, excepting that some one or more do give the Lifschütz test for "oxycholesterol."

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The Halochromism of Ketones in Acids

BY LEIGH C. ANDERSON

Benzophenone, xanthone and many other ketones which are colorless in the pure state give colored solutions when dissolved in sulfuric acid or perchloric acid, a phenomenon which has been designated as halochromism. Many hypotheses, including the quinonoid theory, the oxonium theory, the theory of the "coördinately unsaturated central carbon atom," ionization, etc., have been proposed in order to account for the production of the color of these solutions. The arguments which have been used as evidence in favor of each of these theories have usually been dependent upon the color of the solution rather than upon absorption spectra, and as a result many contradictory conclusions have been drawn. Quantitative absorption spectra data from solutions of ketones in sulfuric acid or perchloric acid support the contention that the colored solute which is present in such solutions is produced through the reactivity of the carbonyl group of the ketone. In addition, the data also indicate that quinoidation is, in some instances, a concurrent phenomenon, particularly in those cases where the carbonyl group may become a part of a quinonoid ring.

The absorption curves (Figs. 1 and 2) for ether solutions of benzoquinone, anthraquinone, benzophenone and xanthone[1] disclose absorption bands at frequencies between 2900 and 3300 mm.$^{-1}$ (about 3000 Å.). This is the region in which absorption bands are found in curves for solutions

(1) The apparatus and the methods used for obtaining the quantitative absorption spectra have been described by Anderson and Gomberg, THIS JOURNAL, **50**, 203 (1928); Anderson, *ibid.*, **51**, 1889 (1929).