of higher alcohols boiling at 135–150° obtained from the synthetic methanol process,[7] was refluxed for twelve hours. The liquid was decanted from a small amount of solid material (4.5 g.) and distilled. After removal of the excess alcohols, the carbamate was obtained at 110–125° under 4 mm. pressure. The product was slightly yellow and remained liquid at room temperature. The yield was 137 g. or about 60% of the theoretical.

**Ethylene Glycol and Urea.**—A mixture of 240 g. of urea, 124 g. of ethylene glycol, and 12 g. of glycerol yielded a transparent water-soluble sirup after a heating period of six hours at 160–165° and four hours at 170–175°.

**Triethylene Glycol and Urea.**—A mixture of 60 g. of urea, 6 g. of glycerol, and 150 g. of triethylene glycol was heated at 140–150° for one and one-half hours and slowly increased during eight hours to 175°. The excess triethylene glycol was removed by distillation and 115 g. (57.8%) of a non-volatile water soluble sirup obtained. Upon standing for two weeks, a small amount of crystalline material separated which, upon crystallization from acetone, melted at 108° and proved to be the diurethan.

(7) The following compounds have been identified in this fraction by Graves, *Ind. Eng. Chem.*, **23**, 1381 (1931): 2-methyl-1-pentanol, 2,4-dimethyl-3-pentanol and 2,4-dimethyl-1-pentanol.

*Anal.* Calcd. for $C_8H_{16}O_6N_2$: C, 40.67; H, 6.77; N, 11.86. Found: C, 40.87; H, 6.65; N, 11.32.

**Sorbitol and Urea.**—Resins of varying viscosity were obtained depending upon the ratio of urea to sorbitol. Pure compounds could not be isolated.

## Summary

*n*-Dodecyl carbamate, octyl carbamate, isobutyl carbamate, and the mixed carbamates from one fraction (b. p. 135–150°) of the higher alcohols obtained in the methanol synthesis have been prepared by the reaction of urea with the corresponding alcohols at atmospheric pressure. Attempts to obtain alkyl carbonates by the same procedure were unsuccessful.

Polyhydric alcohols reacted with urea under the same conditions to yield sirups from which pure compounds could not be isolated except in the case of triethylene glycol. The latter yielded a sirup from which a small amount of the diurethan of m. p. 108° was obtained.

WILMINGTON, DELAWARE RECEIVED MAY 19, 1938

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[CONTRIBUTION FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF BUFFALO]

# A Critical Examination of the Reaction of Iodine Monobromide with Cholestenone and β-Cholestanone

BY J. O. RALLS

In a previous study,[1] it was shown that cholestenone was one of the products of the reaction of iodine monobromide with cholesterol. It was also shown that cholestenone behaved toward halogen in a manner that was then considered "abnormal." In addition, Copping[2] had stated that cholestenone did not yield analytically correct iodine numbers when Dam's[3] suggested application of the Rosenmund and Kuhnhenn[4] method was used. We were interested, therefore, in attempting to ascertain the causes of this peculiarity.

Inasmuch as $\Delta^{4,5}$-cholestenone, commonly called cholestenone (suggested name, coprostenone),[5] contains a double bond and a ketone group (in a conjugated system), the problem was one of attempting to evaluate the role of each in the reaction of cholestenone with iodine monobromide. Cholestanone, which contains no double bond, and cholestanoxime, which contains neither the double bond nor the ketone group, served to evaluate the effect of the carbonyl group; while cholestenoxime, which does not contain the ketone group but still possesses the double bond, was used in attempting to evaluate the role of the latter group.

In the course of the experimentation, hydrogen bromide catalysis of halogenation was investigated, as was, also, the effect of the oximino hydroxyl upon the halogenation of the ethylene group in cholestenoxime. In the latter work, the factors of solvent nature, of air (oxygen) inhibition, and of the configuration of the oxime were considered. In these studies, *syn*-styrylphenylketoxime, 3,5-diphenylisoxazol, and 3,5-diphenylisoxazoline were also used.

## Discussion and Results

The peculiarities of the reaction of cholestenone with iodine monobromide (Curves 1, Fig. 1) are especially evident in the graph of the organic

(1) Ralls, THIS JOURNAL, **55**, 2083 (1933).
(2) Copping, *Biochem. J.*, **22**, 1142 (1928).
(3) Dam, *Biochem. Z.*, **152**, 101 (1924).
(4) K. W. Rosenmund and W. Kuhnhenn, *Z. Untersuch. Nahr. u. Genussm.*, **46**, 154–9 (1923).
(5) O. Rosenheim and H. King, *Ann. Rev. Biochem.*, **3**, 90 (1934).

halogen. There appears to be some autocatalysis, but it is not simple. Inasmuch as cholestenone contains both a double bond and a ketone group, the complexity might well have been due to the influence of these two groups. Because it contained the ketone group only, β-cholestanone was allowed to react with iodine monobromide. It was surprising to find that it consumed halogen more rapidly (Curves 2, Fig. 1) than did cholestenone. (Of course, it has long been known that it did react with halogen.[6,7]) Examination of the curves showed that, while the reaction of cholestanone lacked the aforementioned complexity, it did possess an autocatalytic nature. Doreé[6] has remarked on the initial lag followed by a rapid consumption of halogen accompanied by a marked evolution of halogen acid when cholestanone reacted with bromine. The above author suggested that substitution had occurred. In the work reported here, the ratios between the total halogen consumed and the halogen organically bound were two to one, which fact is indicative of substitution. But β-cholestanol, from which β-cholestanone is derived, did not undergo substitution under similar conditions.[1] Furthermore, substitution, in the common sense, would not be expected to display signs of autocatalysis. But a substitution preceded by an enolization or activation of a ketone group could do so. It is generally conceded that enolization or activation of a saturated ketone precedes its bromination.

The specific evidence that β-cholestanone also undergoes enolization or activation in its reaction with iodine monobromide is the following. First: when cholestanoxime was allowed to react with iodine monobromide, no appreciable reaction occurred (broken Curve 2, Fig. 1). Of course, this is not absolute evidence that the bromination of cholestanone is via enolization, because the presence of the oximino group in cholestanoxime might have changed the activity of nearby hydrogen. Second: determinations of the order of the reaction of cholestanone with iodine monobromide disclosed that it was monomolecular. (Table I is presented as an example of the results—other initial concentrations were also used.) This finding paralleled those of Meyer,[8] who studied the order of the bromination of malonic acid, and of Cohen,[9] who studied the bromination of acetone

(6) Doreé, *J. Chem. Soc.*, **95**, 648 (1909).
(7) Butenandt and Wolff, *Ber.*, **68**, 2091–2094 (1935).
(8) Meyer, *Ann.*, **380**, 212 (1911); *Ber.*, **44**, 218 (1911).
(9) Cohen, THIS JOURNAL, **52**, 2827–2835 (1930).

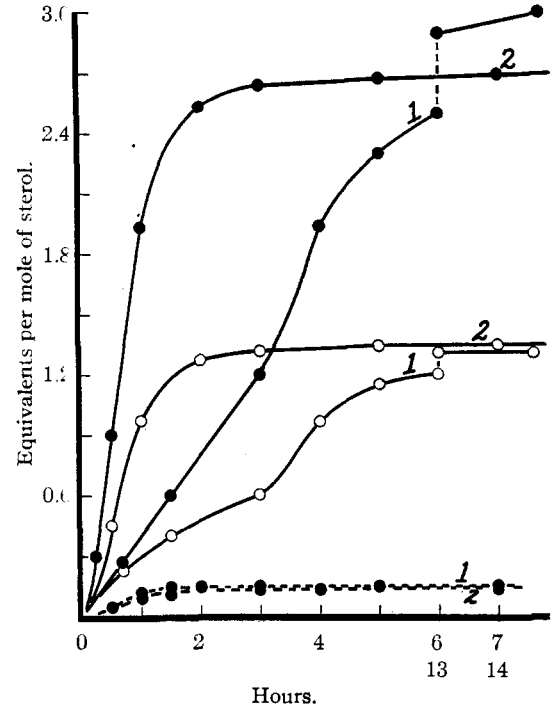



Fig. 1.—The reaction of iodine monobromide with cholestenone, cholestanone, and their oximes: 1, cholestenone; 2, cholestanone; —●—, oximes; ●, total halogen consumed; ○, organic halogen.

in organic solvents. Third: the initial speed of the reaction of cholestanone with iodine monobromide was increased when hydrogen bromide was added to the halogenating agent. These results were similar to those obtained in a like study of cholestenone (Figs. 2 and 3). On the basis of the work of Lapworth,[10] Meyer,[8] Dawson,[11] Cohen,[9] Hanson and Williams,[12] and of Kröhnke,[13] it is generally conceded that mineral acids catalyze the enolization or activation which precedes the bromination of ketones. Further, it is evident that such workers as Butenandt and Wolff[7] and Ruzicka, *et al.*,[14] tacitly assume that cholestanone enolizes during its bromination. Fourth: photographic records of the ultraviolet absorption spectra of cholestanone in chloroform, in chloroform–glacial acetic acid, and in chloroform–glacial acetic acid–hydrogen bromide (Plate 1) and of the enol ethyl ether of cholestanone (Plate 2) in chloroform were made. That of the cholestanone in the presence of hydrogen

(10) Lapworth, *J. Chem. Soc.*, **85**, 30 (1904).
(11) Dawson, *ibid.*, **95**, 1860 (1909); **101**, 1503 (1912); **105**, 532 (1914).
(12) Hanson and Williams, *ibid.*, 1059–63 (1930).
(13) Kröhnke, *Ber.*, **69B**, 921–935 (1936).
(14) L. Ruzicka, Bosshard, Fischer and Wirz, *Helv. Chim. Acta*, **19**, 1147 (1936).

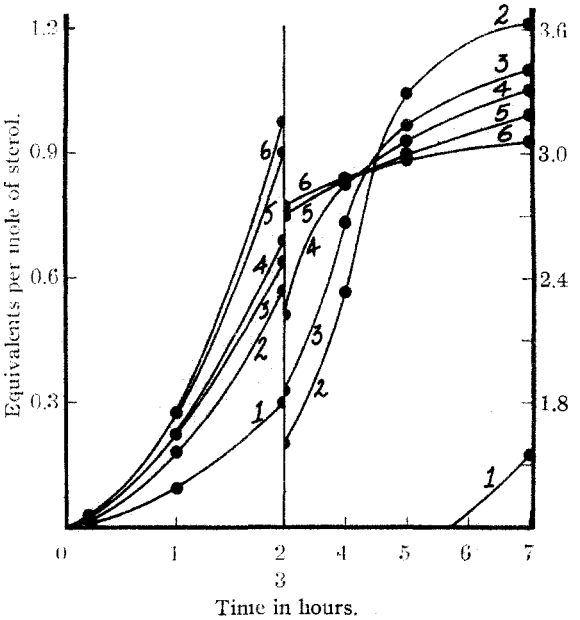



Fig. 2.—The influence of aqueous hydrogen bromide upon the consumption of halogen by cholestenone. Initial hydrogen bromide concentrations mμ 6 cc.: 1, 0.000; 2, 0.020; 3, 0.042; 4, 0.059; 5, 0.079; 6, 0.099.

bromide was similar to that of the enol ethyl ether in chloroform.

TABLE I

THE ORDER OF REACTION OF CHOLESTANONE WITH IBr

Cholestanone in $CCl_4$, IBr in $CH_3COOH$

| Reaction time, min. | $C$ = 0.082 m$M$ Milliequivalents Residual | Con-sumed | Equiv. per mole | $C$ = 0.041 m$M$ Milliequivalents Residual | Con-sumed | Equiv. per mole |
|---|---|---|---|---|---|---|
| Blank | 0.1648 | 0.0000 | 0.000 | 0.0824 | 0.0000 | 0.000 |
| 2 | .1408 | .0240 | .300 | .0704 | .0120 | .300 |
| 4 | .1243 | .0405 | .493 | .0624 | .0200 | .488 |

Order is apparently monomolecular, since $t_2/t_1 = 1 = 2^0 = 2^{n-1}$.

Cholestanone in $CCl_4$, IBr in $CCl_4$

| Reaction time, min. | $C$ = 0.0764 m$M$ Milliequivalents Residual | Con-sumed | Equiv. per mole | $C$ = 0.0382 m$M$ Milliequivalents Residual | Con-sumed | Equiv. per mole |
|---|---|---|---|---|---|---|
| Blank | 0.1528 | 0.0000 | 0.000 | 0.0764 | 0.0000 | 0.000 |
| 2 | .1457 | .0071 | .093 | ... | ... | .. |
| 4 | .1075 | .0453 | .594 | .0536 | .0228 | .597 |
| 8 | .0960 | .0568 | .743 | .0480 | .0284 | .743 |
| 16 | ... | ... | ... | .0440 | .0324 | .849 |

Order is apparently monomolecular.

An error of 0.010 cc. in titration produces a minimum error of 0.010 in equivalents per mole when $C$ = 0.080 m$M$, and one of 0.020 when $C$ = 0.040 m$M$.

While our experiments were being carried on, Inhoffen[15] reported that cholestenone enolizes during its bromination and that hydrogen bromide stimulates the enolization. We, also, had noted that hydrogen bromide catalyzed the reaction of cholestenone with iodine monobromide

(15) Inhoffen, *Ber.*, **69B**, 2141-7 (1936).

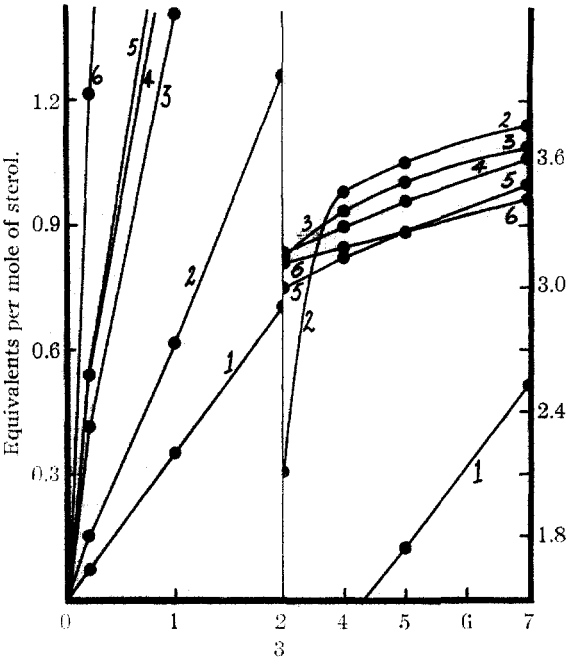



Fig. 3.—The influence of anhydrous hydrogen bromide upon the consumption of halogen by cholestenone. Initial hydrogen bromide concentrations, mμ in 6 cc.: 1, 0.000; 2, 0.020; 3, 0.042; 4, 0.059; 5, 0.079; 6, 0.099.

(Figs. 2 and 3). Further, the reaction of cholestenone with iodine monobromide in carbon tetrachloride-glacial acetic acid, and in carbon tetrachloride alone, was found to be monomolecular. These results are similar to those obtained using cholestanone. Cholestenone reacted similarly to cholestanone in one other respect. Cholestenoxime, like cholestanoxime, resisted halogenation in the presence of glacial acetic acid (Fig. 1 and Table II). (Added hydrogen bromide did not

TABLE II

EQUIVALENTS OF HALOGEN CONSUMED PER MOLE OF SUBSTANCE REACTING WITH IODINE MONOBROMIDE FOR NINETY MINUTES

| Substance | In $CCl_4$ + $CH_3COOH$ In air | In $H_2$ | In $CCl_4$ In air | In $H_2$ |
|---|---|---|---|---|
| Cholestanone | 2.37 | 2.49 | 3.49 | 4.10 |
| Cholestanoxime | 0.11 | 0.13 | 0.57 | 0.63 |
| Cholestenone | .43 | .45 | 0.60 | 0.80 |
| Cholestenoxime | .15 | .15 | 1.17 | 1.24 |
| Cholestenoxime[a] | ... | ... | 0.80 | ... |
| Cholestenoxime[b] | ... | ... | 1.20 | ... |
| Benzalacetophenone | ... | ... | 1.70 | ... |
| *syn*-Styrylphenylketoxime | ... | | 1.42 | 1.42 |
| 3,5-Diphenylisoxazol | ... | | 1.35 | 1.37 |
| 3,5-Diphenylisoxazoline | ... | | 0.11 | 0.11 |

[a] Cholestenoxime, recrystallized from glacial acetic acid. [b] Cholestenoxime, recrystallized from glacial acetic acid and then heated to 120°.

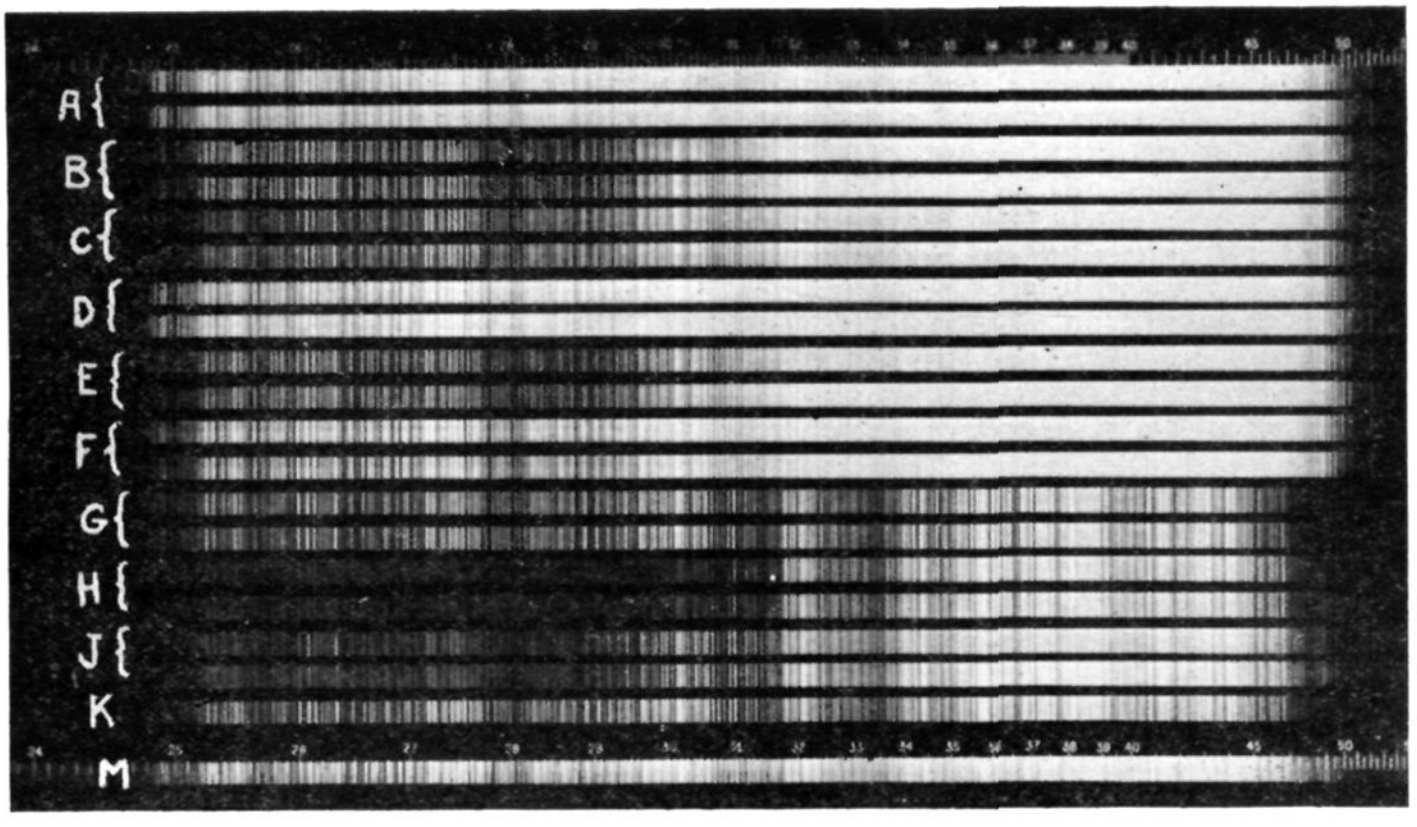



Plate 1.—Ultraviolet absorption spectra of cholestanone in various solvents: A, chloroform control; B, 0.02 *M* cholestanone in chloroform; C, 0.01 *M* cholestanone in chloroform; D, chloroform in 0.04 *M* glacial acetic acid; E, 0.02 *M* cholestanone in chloroform—0.04 *M* glacial acetic acid; F, 0.01 *M* cholestanone in chloroform—0.04 *M* glacial acetic acid; G, chloroform—0.04 *M* glacial acetic acid—0.01 *M* anhydrous hydrogen bromide; H, 0.02 *M* cholestanone in same solvent; J, 0.01 *M* cholestanone in same solvent; K, like J but with 2 × exposure; M, chloroform control.

induce any greater reaction.) This fact was further circumstantial, but not absolute, evidence that cholestenone enolizes during halogenation.

The lack of appreciable reaction between cholestenoxime and iodine monobromide in glacial acetic acid–carbon tetrachloride indicated that, at least in that medium, the double bond of cholestenoxime was not "active." Inhoffen[15] has shown that the ethylene linkage of cholestenone resists bromination and exerts only a directive influence. It seemed to us that the simple statement, *the double bond is not active*, was not sufficient in the case of cholestenoxime. Accordingly, other factors were investigated. Our cholestenoxime was real, or normal, and was not the by-product described by Diels and Abderhalden,[16] for it melted at 151° and did not reduce Fehling's reagent. Air (oxygen) inhibition[17] was not involved (Table II). The nature of the reaction medium was found to affect the extent of reaction (at the end of the arbitrarily selected ninety minutes) of cholestanone, of cholestanoxime, of cholestenone, and of cholestenoxime. The effect was most marked in the case of the last substance and was paralleled by the effect upon the reactions of styryl phenyl ketone and *syn*-styryl phenyl ketoxime with iodine monobromide (Table II). There were five (at least) possible explanations for this marked difference: first, only the rates of reaction in the two media dif-

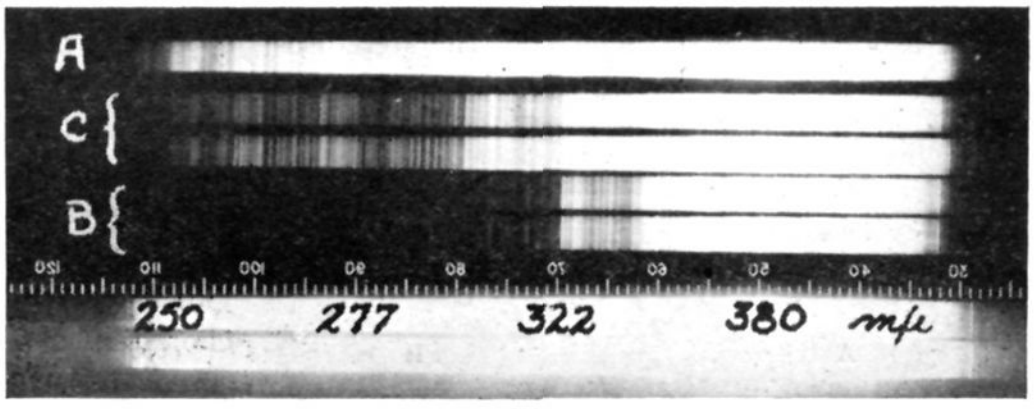



Plate 2.—Ultraviolet absorption spectra of enol ethyl ether of cholestanone: A, chloroform control; B, 0.02 *M* enol ethyl ether of cholestanone (based on the molecular weight of cholestanone) in chloroform; C, 0.01 *M* enol ethyl ether of cholestanone in chloroform.

(16) Diels and Abderhalden, *Ber.*, **37**, 3101 (1904).
(17) Bauer and Daniels, THIS JOURNAL, **56**, 2014 (1934).

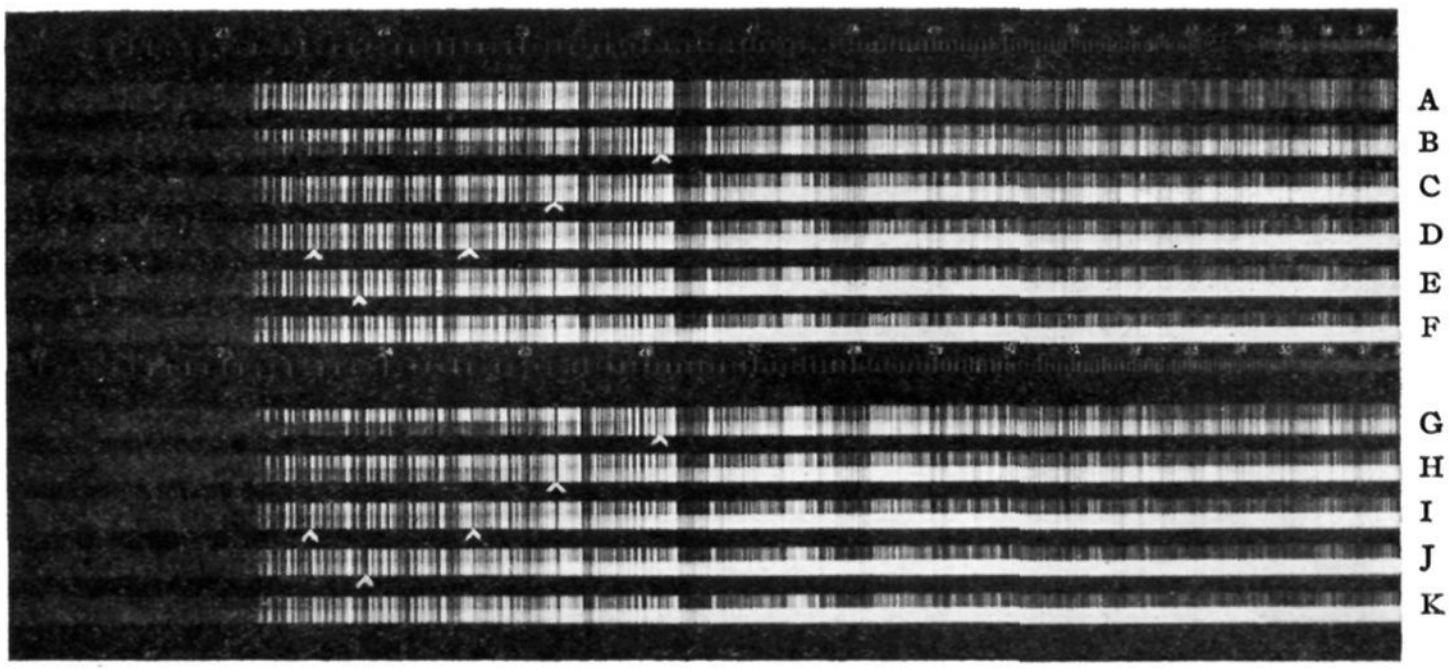
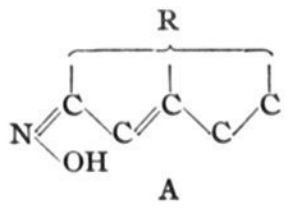
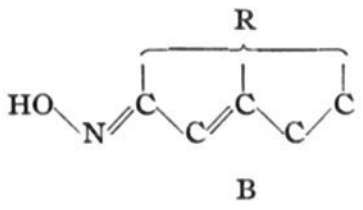



Plate 3.—Ultraviolet absorption spectra of "cholestenoxime" (after crystallization from glacial acetic acid) and of cholestenoxime: solvent, absolute alcohol; tube length 1 cm.; concn., 0.002% or $5 \times 10^{-5}$ molar. Upper half of each spectrograph is that of the controlled or measured light; lower half is that of experimental substance. "Cholestenoxime:" A, control at $d = 0$; B, $d = 0.6$; C, $d = 1.0$; D, $d = 1.2$; E, $d = 1.40$; F, $d = 1.5$. Cholestenoxime: G, $d = 0.6$; H, $d = 1.0$; I, $d = 1.2$; J, $d = 1.4$; K, $d = 1.5$.

fered;[18] second, in the presence of glacial acetic acid, cholestenoxime might have rearranged to Diels and Abderhalden's by-product,[16] but this was not likely; third, glacial acetic acid might have added to the double bond—not at all probable; fourth, in glacial acetic–carbon tetrachloride, cholestenoxime might have undergone cyclization to produce an isoxazoline,[19,20] which cyclization would not occur in carbon tetrachloride alone; fifth, in glacial acetic–carbon tetrachloride, the stereoisomeric cholestenoxime A might have existed, whereas in carbon tetrachloride, B might have existed. In A, due to the proximity of the two groups, coördination between the oximino hydroxyl and the ethylene linkage might have retarded halogenation. In B, the relations would not favor such action.




A




B

When cholestenoxime was dissolved in glacial acetic–carbon tetrachloride and recrystallized from that medium, crystals were obtained which differed in form from those of the original oxime and melted at 60°, resolidified around 90°, and remelted at 151.8° (m. p. of cholestenoxime). The new compound lost weight when it was heated to 90° or more, but this loss was too small for it to have been due to loss of solvent of crystallization. Since the nitrogen content of the compound was the same as that of cholestenoxime, the substance was not an addition product of acetic acid and cholestenoxime. It did not reduce Fehling's reagent, and so was not Diels and Abderhalden's[16] by-product. Its ultraviolet absorption spectrum appeared to be the same as that of the original cholestenoxime (Plate 3). Therefore, it was probably not an isoxazoline (the absorption spectrum of an isoxazoline should differ markedly from that of an $\alpha,\beta$-unsaturated ketoxime). The apparent identity of the ultraviolet absorptions of the new compound and the cholestenoxime, together with the evident ease of converting the one into the other (after being heated to 120°, the new compound did not depress the melting point of cholestenoxime), indicated that the two were probably stereoisomeric ketoximes. When the new compound was allowed to react with iodine monobromide in carbon tetrachloride, it did so more slowly than did either the heat transformed substance or the original cholestenoxime (Table II). Unfortunately, in the only case on record where two stereoisomeric ketoximes have been halogenated, no statement concerning the relative speeds of the halogenations was made.[21] Moreover, the same product (*syn-*

(18) Böeseken and Gelber, *Rec. trav. chim.*, **46**, 158 (1927).
(19) Blatt, THIS JOURNAL, **53**, 1137 (1931).
(20) Auwers and Seyfried, *Ann.*, **484**, 201–2 (1930).
(21) Blatt and Stone, THIS JOURNAL, **53**, 4137 (1931).

dibromostyryl *p*-bromophenyl ketoxime) resulted from the action on both isomers.

Although increased initial concentrations of hydrogen bromide increased the initial rate of halogenation, these greater concentrations depressed the total amount of halogen consumed by cholestenone and by cholestanone (Fig. 2). Such phenomena, at least in other reactions,[22] are usually due to not less than two opposing forces. In these studies, the increased rates were due to the usual catalyzation of enolization. The repression of the halogenation observed at the longer reaction times (four or more hours) was due to the action of the hydrogen bromide but not to that of water, although water did repress the halogenation over its entire course[9] (Figs. 2 and 3). There appeared to be no differences between the general nature of the reactions at low and that at high hydrogen bromide concentrations: monomolecular at 2.5 and at 12.5 millimol. of hydrogen bromide per liter of reaction mixture. A clue as to the probable nature of the repressing action was seen in the fact that, at the lower hydrogen bromide concentration, only 45% of the reacted halogen was organically bound, whereas, at the higher concentration, 55% was so bound. According to Inhoffen,[15] the mechanism of the bromination of cholestenone is: 1, enolization; 2, bromine addition; 3, cleavage of halogen acid; 4, allylation; 5, enolization; 6, bromine addition; 7, halogen acid cleavage; 8, etc. Up to step 3, the product is an unstable dibromide. A stable dibromide results from the whole process. If the hydrogen bromide had partially inhibited step 3, the result would have been a diminished consumption of halogen but a larger proportion of that consumed would have been organically bound. On the other hand, direct addition of hydrogen bromide to the cholestenone in stage 1 could have produced the same observed relations. However, no halogenated steroid was obtained when, in the absence of iodine monobromide, cholestenone was treated with an extremely high concentration of hydrogen bromide.

It is well known that hydrogen bromide, iodine, and bromine interact to form complexes such as $I_2Br^-$, $IBr_2^-$, $IBr_3$, $Br_3^-$, and others. Of these $I_2Br^-$ forms the least readily.[23,24] Transmission measurements (Fig. 4) showed that, in the medium we employed in our studies, a relatively low concentration of hydrogen bromide produced evidence of complex formation with iodine monobromide and with bromine, but that there was little evidence of iodine–$Br^-$ complexes unless the hydrogen bromide concentration was relatively large. Furthermore, by other means, it was found that bromine was the active agent in iodine monobromide, a fact recently reported by Militzer also[25] (Fig. 5). Thus it was evident that an increase in the initial concentration of hydrogen bromide, while stimulating enolization, was also decreasing the concentration of the active halogenating agent. This latter action could have caused the diminished consumption of halogen.

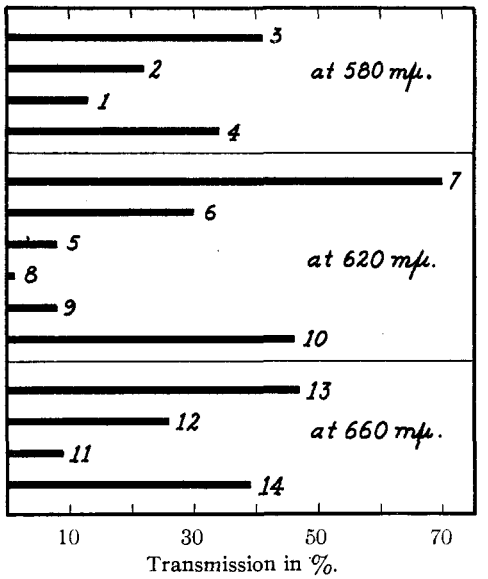



Fig. 4.—Light transmission through bromine, iodine, and iodine monobromide solutions with and without hydrogen bromide: solvent, glacial acetic acid; 1, 0.04 *N* bromine; 2, 0.03 *N* bromine; 3, 0.02 *N* bromine; 4, 0.04 *N* bromine–0.0232 *M* HBr; 5, 0.005 *N* iodine; 6, 0.0025 *N* iodine; 7, 0.0013 *N* iodine; 8, 0.01 *N* iodine–0.0058 *M* HBr; 9, 0.005 *N* iodine–0.0029 *M* HBr; 10, 0.005 *N* iodine–0.0261 *M* HBr; 11, 0.04 *M* iodine monobromide; 12, 0.02 *M* iodine monobromide; 13, 0.01 *M* iodine monobromide; 14, 0.04 *M* iodine monobromide–0.0232 *M* HBr.

### Reaction Products

We did not isolate any of the products of the reaction of iodine monobromide with cholestenone, but, inasmuch as bromination resulted from the treatment with iodine monobromide, they, undoubtedly, would have been the same as those

(22) Nicholson and Holly, Jr., *Ind. Eng. Chem.*, **30**, 114 (1938).
(23) Forbes and Faull, Jr., THIS JOURNAL, **55**, 1820 (1933).
(24) Faull, Jr., *ibid.*, **56**, 522 (1934).
(25) Militzer, *ibid.*, **60**, 256 (1938).

described by Inhoffen[15] and by Butenandt, *et al.*[26,27]

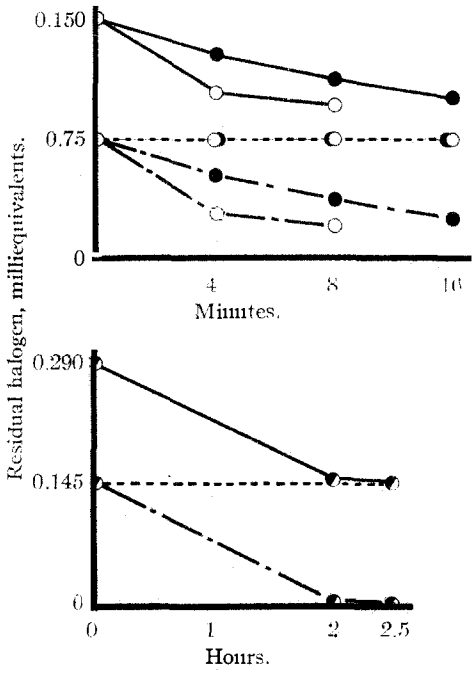
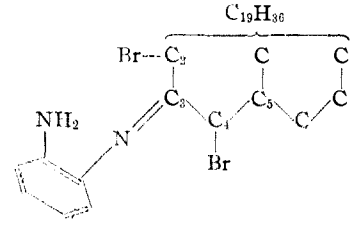



Fig. 5.—Residual total halogen, iodine, and bromine at certain periods in the reaction of iodine monobromide with cholestanone, with 2-bromocholestanone, and with cholestenone (solvent, carbon tetrachloride): ○, cholestanone; ●,2-monobromocholestanone; ◐, cholestenone; – – – – – –, iodine; – – – –, bromine; ———, total halogen.

From β-cholestanone-iodine monobromide reaction mixtures, three compounds were isolated: a monobromide, m. p. 171.5°; and two dibromides, m. p. 147 and 194°, respectively. The monobromide proved to be 2-monobromocholestanone.[7] Although the one dibromide (m. p. 147°) has not, as yet, been identified, it must contain at least one bromine atom at position 2 of the steroid structure, for it was a product of the reaction of iodine monobromide with 2-monobromocholestanone also.

The other dibromo compound (m. p. 194°) has been the subject of much debate. Ruzicka, *et al.*,[14] maintained that it was 2,2-dibromocholestanone, while Butenandt, *et al.*,[26] identified it as 2,4-dibromocholestanone. Lately, the latters' findings have been confirmed.[28] Although we firmly believe that the compound is 2,4-dibromocholestanone, we must admit that we were unable to get a diosphenol by treating it with potassium acetate.[26] On the other hand, the compound did yield a derivative with *o*-phenylenediamine, but this derivative was not the quinoxaline described by Ruzicka,[14] although it did have the same melting point (184°). It still contained halogen. Inasmuch as one bromine in the dibromo compound is known to be at position 2, and in view of the fact that the nitrogen and the halogen content of the *o*-phenylenediamine derivative were such as to indicate the presence of two atoms of each, it is believed that this *o*-phenylenediamine derivative was 3-*o*-aminoanilido-2,4-dibromocholestane, m. p. 184°, $C_{33}H_{50}N_2Br_2$, or




## Materials

**Cholestenone,** $\Delta^{4,5}$, m. p. 81°, prepared according to Windaus[29] and Schoenheimer.[30]

**Cholestanone,** m. p. 129.5°, according to Doreé.[6]

**Cholestenoxime,** m. p. 151°; and *cholestanoxime,* m. p. 199°, according to Diels and Abderhalden.[16]

**2-Monobromocholestanone,** m. p. 171.5°.[7]

**Benzalacetophenone,** m. p. 57–58°, a recrystallized Eastman Kodak Co. product.

*syn*-**Styryl phenyl ketoxime,** m. p. 116°,[19,21] prepared as described by Henrich.[31]

**3,5-Diphenylisoxazol,** m. p. 140°,[32,33] prepared according to Goldschmidt.[34]

**3,5-Diphenylisoxazoline,** m. p. 75°,[19,20,33] prepared as described by Henrich.[31]

## Experimental

**The Reaction of Iodine Monobromide with Cholestenone, with Cholestanone, with Cholestenoxime, and with Cholestanoxime.**—Twenty cc. of 0.0167 *M* steroid in carbon tetrachloride was pipetted into one arm of a T-tube (turned on its side), while 40 cc. of approximately 0.08 *N* iodine monobromide in glacial acetic acid was measured accurately into the foot of the T-tube. After the two solutions had come to temperature (25°), they were mixed quickly. At the end of definite intervals of time, 6-cc. aliquot portions were removed for analysis. Residual or

(26) Butenandt, Schramm, Wolff and Kudzus, *Ber.*, **69B**, 2779–2783 (1936).

(27) Butenandt, Schramm and Kudzus, *Ann.*, **531**, 176–208 (1937).

(28) Inhoffen, *Ber.*, **70**, 1695 (1937).

(29) Windaus, *ibid.*, **39**, 518 (1906).

(30) Schoenheimer, *J. Biol. Chem.*, **110**, 461 (1935).

(31) Henrich, *Ann.*, **351**, 172 (1907).

(32) Fleck, Dissertation, Leipzig, 1903.

(33) Auwers and Muller, *J. prakt. Chem.*, **137**, 57, 81 (1933).

(34) Goldschmidt, *Ber.*, **28**, 986 (1895).

unreacted halogen, halogen acid, and organic halogen were determined as previously described.[1] The total halogen consumed and the organically bound halogen are plotted in Fig. 1, as equivalents of halogen per mole of sterol, against reaction time in hours.

**The Order of the Reaction of Cholestenone, of Cholestanone, and of 2-Monobromocholestanone with Iodine Monobromide.**—In determining the order of reaction, we employed the method described by Lewis[35] in which the time required for any fraction of the initial concentration (the initial concentrations of all reacting components are made equal) of a substance to react is compared with the time required for the same fraction to react when the initial concentration is different from the first. By this method, $n = 1 + \frac{\log t_2/t_1}{\log a_1/a_2}$, where $n$ is the order of the reaction, $t_1$ and $t_2$ are the respective reaction times, and $a_1$ and $a_2$ are the respective initial concentrations. If $a_1$ is made equal to $2a_2$, then $n = 1 + \frac{\log t_2/t_1}{\log 2}$, or $2^{n-1} = t_2/t_1$.

In actually making the determinations, the steroid solution was measured into a 200 × 25 mm. test-tube and the iodine monobromide was measured from a microburet into a thin-walled glass bulb. The bulb was carefully slid into the tube and then quickly broken by means of a glass rod passed through the stopper used to close the tube. At the expiration of one or two or four or eight, etc., minutes, 3 cc. of 0.05 *M* potassium iodide was added and the unreacted halogen was titrated with standard sodium thiosulfate. As an example of the results, a sample of those for cholestanone are given in Table I. (The notation $C = 0.082\ mM$ means that there were that many milliequivalents of each reactant in the mixture.) The reaction was first order for each of the substances tested.

**The Effect of Hydrogen Bromide upon the Reaction of Iodine Monobromide with Cholestenone, with Cholestanone, and with 2-Monobromocholestanone.**—These reactions were run and the results obtained in a manner similar to that used for the study of the reaction of iodine monobromide with cholestenone and cholestanone, except that varying initial concentrations of hydrogen bromide were secured by adding definite quantities of aqueous 48% hydrogen bromide, or anhydrous hydrogen bromide in glacial acetic acid, to the iodine monobromide solutions. The results of the studies on cholestenone are plotted as equivalents of halogen consumed per mole of steroid (Figs. 2 and 3). The effect upon the reactions with cholestanone and 2-monobromocholestanone was the same except for slight differences in degree.

**The Ultraviolet Absorption Spectrum of Cholestanone as Affected by Hydrogen Bromide.**—Two hundredths molar (0.02 *M*) solutions of cholestanone in chloroform, in 0.04 *M* glacial acetic acid in chloroform, and in 0.04 *M* glacial acetic acid and 0.01 *M* anhydrous hydrogen bromide in chloroform were prepared. Diluting solutions like those above, but not containing the steroid, were also prepared. Using a 25-mm. absorption cell, photographs were made of the spectra of each solvent and of 0.02 and of 0.01 *M* cholestanone (Plate 1). Photographs were made of the spectra of chloroform and of 0.02 and 0.01 *M* enol ethyl ether of cholestanone in chloroform[36] (Plate 2). Unfortunately, the instrument used in the first part was not available for this second work.

**The Reaction of Iodine Monobromide with Certain Substances as Affected by: (a) Nature of Solvent, (b) Air (Oxygen) and Hydrogen.**—Cholestenone, cholestenoxime, cholestanone, cholestanoxime, benzalacetophenone, *syn*-styryl phenyl ketoxime, 3,5-diphenylisoxazol, 3,5-diphenylisoxazoline, and a product derived from cholestenoxime, by treatment with glacial acetic acid, were the materials used in these experiments. In each case, 0.033 mmol. of the substance was weighed accurately and transferred to a 200 × 32 mm. test-tube. Two cc. of carbon tetrachloride was added. The tube was closed with a two-holed rubber stopper carrying a glass stopcock and a glass tube closed by means of pinch-clamp on a rubber tube. The test-tube was evacuated and filled with hydrogen three times. Then 4 cc. of 0.04 *M* iodine monobromide in glacial acetic acid or in carbon tetrachloride was admitted. After ninety minutes, the unreacted halogen was titrated with standard thiosulfate. These experiments, but with the omission of the evacuation and filling with hydrogen, were repeated (see Table II).

**The Effect of Glacial Acetic Acid upon Cholestenoxime.**—Two hundred milligrams of cholestenoxime was dissolved in 12 cc. of carbon tetrachloride and 24 cc. of glacial acetic acid. After one hour, the solution was concentrated *in vacuo* at a low temperature. Upon chilling the concentrate, crystals separated which were filtered, washed with cold alcohol and cold water, and dried by suction. These crystals melted at 60°, resolidified at 90°, and then remelted at 151.8° (the latter is the melting point of cholestenoxime). When weighed samples were heated to 120° and then reweighed, they were found to have lost 1.85% of their weight. The calculated loss of weight for solvent of crystallization would have been: for 1 $CH_3COOH$, 13.0%; for 1 $C_2H_5OH$, 10.3%; for 1 $H_2O$, 4.2%; and for ½ $H_2O$, 2.2%.

*Anal.* Calcd. for $C_{27}H_{45}ON$ (oxime or isoxazoline): N, 3.5. Found: N, 3.5, 3.52.

The absorption spectrographs of 0.002% solutions of cholestenoxime and of the above product from glacial acetic acid displayed the same maximum at 238 mμ ($\log \epsilon = 4.4$) (Plate 3).

Two samples of crystals, described above (the one sample was heated to 120° and cooled, while the other was not so treated), were allowed to react with iodine monobromide. The untreated material consumed less halogen than did the other (Table II).

**The Effect of Small and of Large Initial Hydrogen Bromide Concentrations upon the Order of the Reaction of Iodine Monobromide with Cholestenone.**—These experiments were carried out in the same way as the others preceding these, except two different iodine monobromide solutions were used. The one was made 0.005 *M* and the other 0.025 *M*, with respect to hydrogen bromide. The reaction was first order in both solutions.

**The Relation between the Halogen Combined with and the Total Consumed by Cholestenone Reacting with Iodine**

(35) Lewis, "A System of Physical Chemistry," Vol. I, Longmans, Green and Company, New York, N. Y., 1918, pp. 397–8, B.

(36) The author wishes to thank Dr. G. H. Cartledge, of the University of Buffalo, Chemistry Department, for his aid and suggestions in the spectrophotometric work.

**Monobromide Solutions Containing Small and Large Initial Amounts of Hydrogen Bromide.**—The solutions of cholestenone and iodine monobromide were 0.035 *M*. The iodine monobromide solutions were made 0.005 and 0.025 *M*, with respect to hydrogen bromide. At the end of seven hours, total halogen consumed and organically bound halogen were determined as in the first "experimental" section. At seven hours, in the lower concentration of hydrogen bromide, cholestenone had reacted with 0.9 equivalent of halogen per mole of steroid. Of this 0.4 equivalent, or 45%, was organically bound. At the higher concentration of hydrogen bromide, the results were: 0.8 reacted and 0.46, or 55%, bound.

**Attempt to Find Products of the Addition of Hydrogen Bromide to Cholestenone and to Cholestanone.**—Solutions of 0.2 g. of cholestenone and of 0.2 g. of cholestanone in 20 cc. of carbon tetrachloride were prepared. Each solution was "loaded" with 2.5 cc. of 4.27 *N* anhydrous hydrogen bromide in glacial acetic acid. After four hours, the solutions were brought to dryness *in vacuo*. The residues from each were recrystallized from alcohol. No halogen containing products were found.

**The Effect of Added Hydrogen Bromide upon Bromine, upon Iodine, and upon Iodine Monobromide in Glacial Acetic Acid.**—Solutions of bromine, of iodine, and of iodine monobromide in glacial acetic acid were prepared. To portions of each, definite quantities of 4.27 *N* hydrogen bromide in glacial acetic acid were added. (For concentrations, please see the key to Fig. 4.) The transmission of light of various wave lengths was measured through 1 dm. of each solution. The results are shown as a barogram in Fig. 4. Only a few wave lengths are shown in order to avoid complexity.

**Evidence that the Bromine of Iodine Monobromide, and Not the Iodine, nor Iodine Monobromide, Reacts with the Steroid in the Reactions under Investigation.**—Into each of several large test-tubes were pipetted 2 cc. of 0.0167 molar solutions of steroid (cholestanone and 2-monobromocholestanone) in carbon tetrachloride and 2 cc. of 0.0373 *M* iodine monobromide in carbon tetrachloride. In the case of cholestenone, double the indicated volumes were used. At the end of definite intervals of time, total residual halogen was determined in the usual way, while the residual iodine was determined by means of a modification of a procedure for the determination of iodine and bromine in the presence of one another as described by Spitzer.[37] Our procedure was: to the reaction mixture, at the expiration of the reaction period selected, 3 cc. of 3% potassium bromide in water and 0.75 cc. of 5% sodium formate (made from redistilled formic acid) were added and the mixture was shaken moderately for three minutes. This treatment reduced the residual bromine but left the iodine untouched (glacial acetic acid must be absent). Then 3–5 cc. of 0.05 *M* potassium iodide was added and the iodine was titrated in the usual manner. Residual bromine was taken as the difference between the total residual halogen and the residual iodine. The results are plotted as milliequivalents of residual halogen against the time of reaction (Fig. 5). Iodine did not react.

**The Isolation of the Products of the Reaction of Iodine Monobromide with Cholestanone.**—A solution of 4.95 g. of cholestanone in 660 cc. of carbon tetrachloride was mixed with 1320 cc. of 0.04 *M* iodine monobromide in glacial acetic acid. A 6-cc. aliquot of this mixture required 4.37 cc. of 0.0737 *N* thiosulfate for the complete reduction of the halogen. When the titer had fallen to and remained constant at 2.95 cc. (2.7 equivalents of consumed halogen per mole of steroid), water, potassium iodide, and thiosulfate were added to remove the excess halogen. The carbon tetrachloride layer was washed free of acid with plenty of water and sodium bicarbonate. It was then dried and distilled *in vacuo*. The residue was leached with warm alcohol, from which, upon being cooled, crystals separated. Fractionation of these, with methyl alcohol–acetone, yielded three products: A, 3.60 g., m. p. 171.5°; B, 0.08 g., m. p. 147°; and C, 0.15 g., m. p. 194°.

Determination of the halogen content of each of these products was effected by means of the Willard–Thompson method.[38]

*Anal.* Subst. A. Calcd. for $C_{27}H_{45}OBr$: Br, 17.2. Found: Br, 17.9. Subst. B. Calcd. for $C_{27}H_{44}OBr_2$: Br, 29.4. Found: Br, 30.1. Subst. C. Calcd. for $C_{27}H_{44}OBr_2$: Br, 29.4. Found: Br, 30.4.

A is, therefore, monobromo, and B and C are dibromo compounds. It is noteworthy that a small amount of B and a larger amount of C were obtained when A reacted with iodine monobromide under the same conditions that produced A, B, and C from cholestanone.

**The Action of Potassium Acetate upon Compound A** (m. p. 171.5°).—Two-tenths gram of A and 19 cc. of 21% fused potassium acetate were mixed and heated at 200° for five hours. The mixture was then diluted with water and extracted several times with small portions of ether. The combined extracts were washed free of acid, dried, and distilled *in vacuo*. The residue, recrystallized from dilute alcohol, yielded a small amount of fine plates which melted at 111°. This melting point corresponds to that given for $\Delta^{1,2}$-cholestenone which is obtainable by the action of potassium acetate upon 2-monobromocholestanone.[7]

**The Action of Dry Pyridine upon Compound A** (m. p. 171.5°).—Two-tenths gram of A was dissolved in 8 cc. of dry pyridine and refluxed for fifteen hours. When the solution had cooled, 30 cc. of ether was added. The pyridine was washed out with water. The ether was dried and then distilled *in vacuo*. The residue, when recrystallized, proved to be the original starting material. The bromine of A was, therefore, not in position 4 or 5, but rather in position 1 or 2. This is further evidence that A is 2-monobromocholestanone.

**The Action of Fused Potassium Acetate upon Compound B** (m. p. 147°).—One-tenth gram of compound B was dissolved in 3 cc. of benzene and 7 cc. of absolute alcohol. Powdered fused potassium acetate was added and the mixture was allowed to stand at room temperature for forty-eight hours. Ether and water were then added. The ether layer was washed, dried, and distilled *in vacuo*. The residue crystallized from acetone as fine needles (m. p. 119°). The crystals contained no halogen. For want of material, no further work was done on compound B.

**The Action of Fused Potassium Acetate and of Dry Pyridine upon Compound C** (m. p. 194°).—Although compound C was treated with fused potassium acetate and

(37) Spitzer, *Ind. Eng. Chem., Anal. Ed.*, **8**, 465 (1936).

(38) Willard and Thompson, THIS JOURNAL, **52**, 1893 (1930).

with dry pyridine exactly as was A, no definitely crystalline product was obtained. Nor was a definitely pure crystalline product obtained when 650 mg. of compound C was refluxed with 60 cc. of butyl alcohol containing 3 g. of potassium acetate. The impure products, in each case, did not give a typical positive diosphenol reaction with ferric chloride. It did give a dirty brownish-red color, which, possibly, could be called positive.[26]

**The Action of *o*-Phenylenediamine upon Compound C** (m. p. 194°).—A solution of 0.5 g. of C and 0.2 g. of *o*-phenylenediamine in 170 cc. absolute alcohol was refluxed for five hours. The alcohol was then carefully distilled at low temperature *in vacuo*. The residue was dissolved in ether and the excess phenylenediamine was extracted with dilute hydrochloric acid. The ether, after being washed thoroughly, was dried and distilled *in vacuo*. The residue, dissolved in alcohol, yielded crystals which melted at 184°. This was the same as the melting point given for the compound said to be the quinoxaline derived from the purported 2,2-dibromocholestanone.[14] But our derivative still contained halogen.

*Anal.* Calcd. for $C_{33}H_{50}N_2Br_2$: N, 4.41; Br, 25.2. Found: N, 4.34; Br, 25.6.

## Summary

1. The reactions of cholestenone and of cholestanone with iodine monobromide possess autocatalytic characteristics.

2. The reaction of iodine monobromide with cholestenone, with cholestanone, and with 2-monobromocholestanone is first order.

3. The oximes of cholestenone and cholestanone do not react readily with iodine monobromide in glacial acetic acid.

4. As in the case of the action of bromine upon cholestenone, evidence indicates that enolization is the first step in the reaction of cholestenone with iodine monobromide.

5. Hydrogen bromide speeds the action of iodine monobromide upon cholestenone, cholestanone, and 2-monobromocholestanone, but it has no detectable influence on the reaction with the oxime of either cholestenone or cholestanone.

6. The double bond in cholestenoxime is resistant to halogenation in a glacial acetic acid containing medium.

7. There is some evidence that cholestenoxime undergoes a rearrangement in glacial acetic acid.

8. In concentrations of hydrogen bromide greater than a small amount, the total halogen consumed by cholestenone, by cholestanone, and by 2-monobromocholestanone is decreased, but the proportion of organically bound halogen is increased.

9. The inhibiting influence of the larger concentrations of hydrogen bromide may be due to its effect on intermediate steps in the reactions, or to its formation of complexes with bromine or with iodine monobromide.

10. Bromine is the active ingredient of iodine monobromide.

11. 2-Monobromocholestanone, m. p. 171.5°, is a product of the reaction of cholestanone with iodine momobromide.

12. An unidentified dibromo derivative of cholestanone was isolated (m. p. 147°).

13. Further evidence that the dibromocholestanone, melting at 194°, is 2,4-dibromocholestanone, and not 2,2-dibromocholestanone, is given.

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[CONTRIBUTION FROM THE BELL TELEPHONE LABORATORIES]

# An Electron Diffraction Examination of Some Linear High Polymers

BY K. H. STORKS

Because of the general structural similarities between synthetic linear polyesters and many natural fibers, fundamental studies of the simpler compounds are of obvious importance. Several members of a series of these esters have been made the subject of X-ray study by Fuller and Erickson.[1] It is possible to prepare many of these in the form of films suitable for examination by electron diffraction. The experiments to be described here have two purposes: to demonstrate the utility of the electron diffraction method for studies of the structure of highly polymerized materials, and to discover the behavior of macromolecules in extremely thin layers. The methods are shown to be equally applicable to the natural polymer gutta-percha.

The fact that these materials can be cold drawn to a considerable extension without breakage is well known,[2] a high degree of orientation

(1) C. S. Fuller and C. L. Erickson, THIS JOURNAL, **59**, 344 (1937).

(2) W. H. Carothers and J. W. Hill, *ibid.*, **54**, 1579 (1932).