COLOR REACTION OF CHOLESTEROL WITH TRICHLOROACETIC ACID AND ANTIMONY TRICHLORIDE. ON THE REACTION MECHANISM

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Abstract

The color reaction of cholesterol with trichloroacetic acid and antimony trichloride was examined to elucidate its reaction mechanism. 3,5-Cholestadiene, 3,3'-bis(3,5-cholestadiene), 3,3'-bis(2,4-cholestadiene), and cholesteryl trichloroacetate were isolated as the reaction products from the colored reaction mixture of cholesterol, and the first three compounds were found to be responsible for the coloration. It was assumed that cholesterol was dehydrated to 3,5-cholestadiene and 2,4-cholestadiene, which were dimerized to 3,3'-bis(3,5-cholestadiene) and 3,3'-bis(2,4-cholestadiene), respectively, and 3,3'-bis(2,4-cholestadiene) was in part converted to 3,3'-bis(3,5-cholestadiene) in trichloroacetic acid and antimony trichloride. The free radicals were detected in the colored solutions of cholesterol, 3,5-cholestadiene, 3,3'-bis(3,5-cholestadiene), and 3,3'-bis(2,4-cholestadiene), and inferred to be the radical cations of the steroids. The radical cation was postulated to be responsible with respect to the mechanism of the coloration. The relationship between the color reagent and the formation of dimeric steroids was described.

It has been reported that 3,5-cholestadiene (I), 3,3'-bis(3,5-cholestadiene) (II), and 3,3'-bis(2,4-cholestadiene) (III) are isolated in various color reactions of cholesterol with the Brønsted and/or the Lewis acids, and that these compounds were the main products for coloration with the color reagents(1, 2, 3, 4, 5, 6, 7). From the review of extensive work on the reaction mechanism of cholesterol, it was suggested that the formation of the dimer (II) and/or the dimer

(III) varied with the kind of acids employed in the color reactions as follows. The dimer (II) was obtained from the reaction with trichloroacetic acid and hydrochloric acid (10:1) (1); the dimer (III) from the reaction with zinc chloride and acetyl chloride (Tschugaeff reaction) (2), with antimony trichloride and acetyl chloride (3), with ferric chloride and sulfuric acid (Zak-Henly reaction) (4), and with ferric chloride, perchloric acid, and phosphoric acid (5); and the dimers (II) and (III) from the reactions with sulfuric acid and acetic anhydride (Liebermann-Burchard reaction) (6), and sulfuric acid (Salkowski reaction) (7). These results indicate that the dimers (II) and (III) were mainly obtained in the Brønsted acid medium and in the Lewis acid-containing media, respectively. It may also be said that sulfuric acid, which yielded the dimers (II) and (III) as the dimeric steroids in the Liebermann-Burchard and the Salkowski reactions, has both characters of the Brønsted and the Lewis acids. Moreover, Watanabe (6) claimed that 3,5-cholestadiene and 2,4-cholestadiene (IV) should be the intermediates to the dimers (II) and (III), respectively, in the Liebermann-Burchard reaction. Tese findings inferred that the dimers (II) and (III) should be produced simultaneously in the reaction of cholesterol with a mixture of the Brønsted and the Lewis acids, which yielded the dimers (II) and (III), respectively. Therefore, the reaction of cholesterol with trichloroacetic acid and antimony trichloride (TCA-$SbCl_3$) (8) was examined to elucidate its reaction mechanism and the relationship between the color rea-

gents and the formation of dimeric steroids.

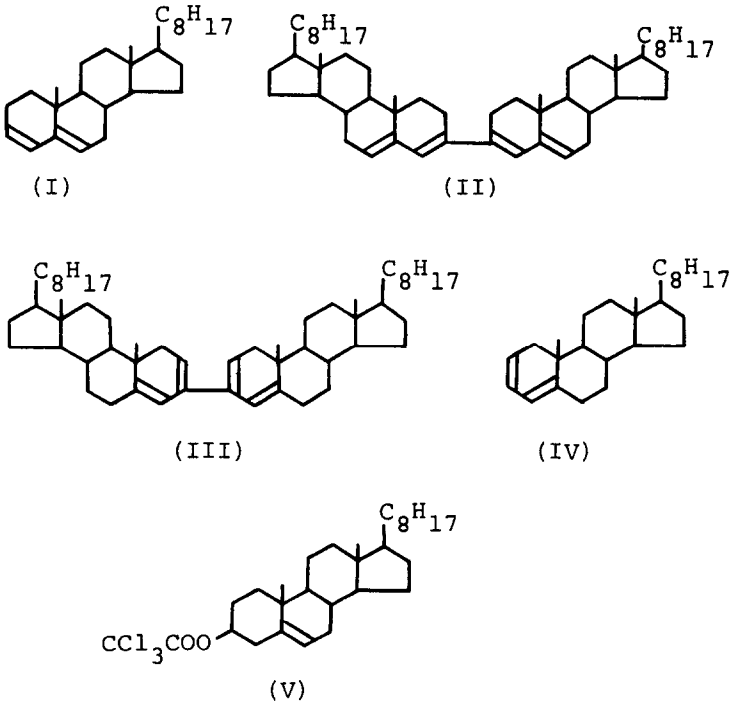

Chart 1

Cholesterol gradually colors yellow, yellowish red, and then red in TCA-$SbCl_3$. The absorption spectra of the colored solution are shown in Fig. 1, and the absorption maxima are observed at 488 nm in the spectrum 1 and clearly at 500 nm in the spectrum 2. The red colored reaction mixture was washed with 10% hydrochloric acid and then water, and the reaction product was extracted with chloroform. The oily residue ob-

tained by the evaporation of chloroform was submitted to column chromatography over silica gel, and four substances were isolated. From the analytical and spectral data, they were identified as 3,5-cholestadiene (I) (9), 3,3'-bis(3,5-cholestadiene) (II) (10), 3,3'-bis(2,4-cholestadiene) (III) (11), and cholesteryl trichloroacetate (V) (1).

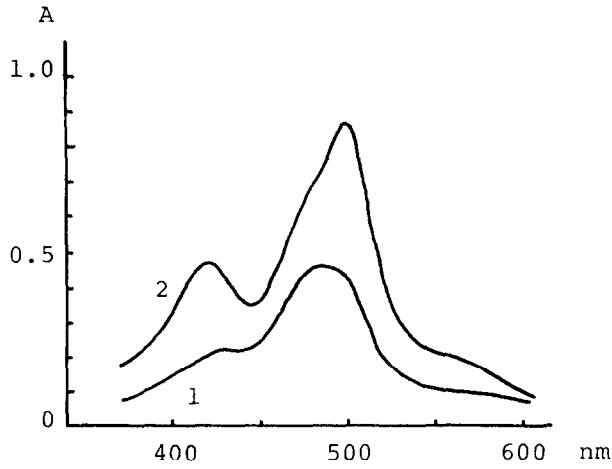

Fig. 1. Absorption Spectra of the Color developed by the Reaction of Cholesterol with TCA-$SbCl_3$

Spectra 1 and 2 were measured at 25 and 53 min, respectively. Concentration of cholesterol was 0.50 mg/ml.

These four compounds isolated were dissolved respectively in TCA-$SbCl_3$ to examine whether they gave rise to coloration similar to that of cholesterol. 3,5-Cholestadiene and the dimer (II) exhibited the immediate red coloration on shaking with TCA-$SbCl_3$, respectively. The absorption spectra of the red colored solution of 3,5-cholestadiene and the dimer (II) are

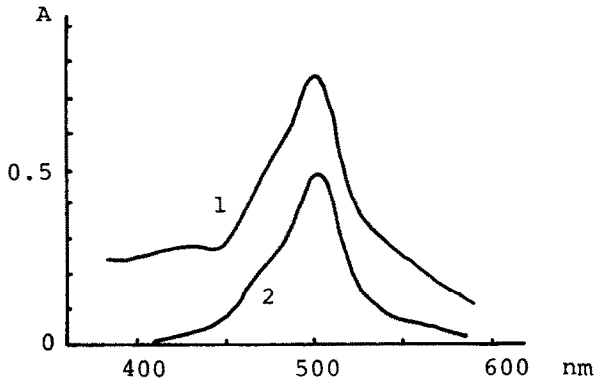

Fig. 2. Absorption Spectra of the Color developed by the Reaction of 3,5-Cholestadiene (1) and 3,3'-Bis(3,5-cholestadiene) (2) with TCA-$SbCl_3$

Spectra 1 and 2 were measured at 6 and 8 min, respectively. Concentration of 3,5-cholestadiene was 0.30 mg/ml and that of 3,3'-bis(3,5-cholestadiene) was 0.0033 mg/ml.

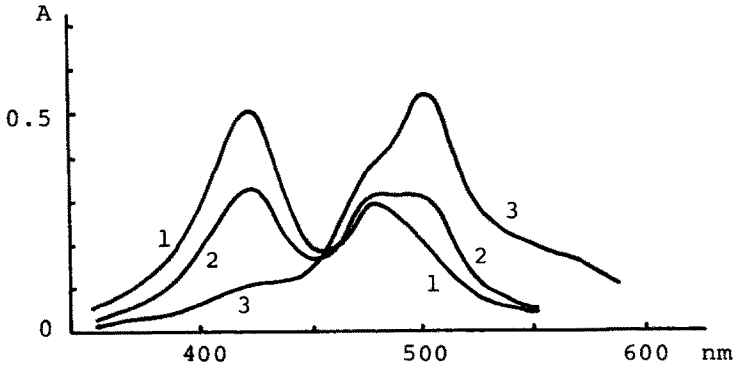

Fig. 3. Absorption Spectra of the Color developed by the Reaction of 3,3'-Bis(2,4-cholestadiene) with TCA-$SbCl_3$

Spectra 1, 2, and 3 were measured at 6, 21, and 71 min, respectively. Concentration of 3,3'-bis(2,4-cholestadiene) was 0.012 mg/ml.

shown in Fig. 2, and the absorption maxima are observed clearly at 500 nm. The dimer (III) colored yellow, yellowish red, and then red on shaking with TCA-$SbCl_3$. The absorption spectra of the colored solution of the dimer (III) are shown in Fig. 3, and the absorption maxima are observed at 420 and 480 nm at the beginning. The absorption maximum at 420 nm gradually decreased and that at 500 nm developed with lapse of time. Cholesteryl trichloroacetate did not show any coloration on shaking with TCA-$SbCl_3$ at a room temperature. From the observation of these absorption spectra, it was found that 3,5-cholestadiene, the dimer (II), and the dimer (III) were responsible for the coloration of cholesterol in TCA-$SbCl_3$, since each of the absorption maxima corresponded to those of cholesterol.

The shift of the absorption maxima from 420 and 480 nm to 500 nm was observed in the spectra of the dimer (III). In order to clarify this mechanism, the dimer (III) was dissolved in TCA-$SbCl_3$ to color for one hour, and the colored solution was treated with 10% hydrochloric acid and then water to afford crude reaction product. Thinlayer chromatography (TLC) (silica gel, hexane or benzene) of this product showed two spots identical with the dimer (II) (Rf=0.63, hexane) and the dimer (III) (Rf=0.79, hexane) in comparison with the authentic samples (10, 11). Moreover, ultraviolet (UV) spectrum exhibited the absorption maxima, which arised from the dimer (II) (λ_{max}, 298, 312, and 328 nm) and the dimer (III) (λ_{max}, 272, 280, and 292 nm). In this spectrum, the absorbance of the maximal peak of the dimer (II) at 312 nm was about 56% against that of the dimer (III) at 280 nm.

From the results, the dimer (III) was confirmed to change into the dimer (II) on the coloration in TCA-$SbCl_3$. The appearance of the absorption maximum at 500 nm in the spectra of the dimer (III) should relate to the conversion of the dimer (III) to the dimer (II).

The dimer (II) was also obtained from the colored reaction mixture of 3,5-cholestadiene with TCA-$SbCl_3$. These findings indicated that the dimer (II) was formed from two pathways, which were the conversion of the dimer (III) and the dimerization of 3,5-cholestadiene in TCA-$SbCl_3$. Formation of the dimer (III) was assumed to be due to the dimerization of 2,4-cholestadiene (3, 6)

On the other hand, free radicals were detected near 3400 gauss in each of the colored solutions of cholesterol, 3,5-cholestadiene, the dimer (II), and the dimer (III) with TCA-$SbCl_3$ by electron spin resonance (ESR) measurement. The g-values of the free radicals are shown in Table I. The free radicals detected were assumed to be the radical cations derived from the steroids and to contributed to the coloration (3,12). It is well known

Table I. g-Values of Free Radicals detected in Colored Solutions of Steroids with TCA-$SbCl_3$

Compound	g-Value
Cholesterol	2.003
3,5-Cholestadiene	2.003
3,3'-Bis(3,5-cholestadiene)	2.003
3,3'-Bis(2,4-cholestadiene)	2.003

that oxygen takes up the hydrogen atom (13, 14, 15, 16) and an electron (13, 14) from the organic compounds in various reaction systems. It may be said that the take-up of the hydrogen atom and an electron is carried out by oxygen in the present investigation, since an oxidizing agent is not present in TCA-$SbCl_3$. However, an experiment was not made to exclude oxygen from TCA-$SbCl_3$ so as to examine whether it is indispensable for the formation of radical cations and the resulting coloration, because it was evident that TCA, even if free from oxygen, gave rise to coloration by the protonation of steroids possessing conjugated double bonds (4, 6, 17). From the findings given above, it was postulated that the reaction proceeded as shown in Chart 2.

Our previous paper (3) reported that the reaction of cholesterol with antimony trichloride and acetyl chloride gave 2,4-cholestadiene, which was in part dimerized to the dimer (III), and that 3,5-cholestadiene did not exist in the colored solution of cholesterol and consequently the dimer (II) was not isolated. On the other hand, the dimers (II) and (III) were simultaneously isolated from the colored reaction mixture of cholesterol with TCA-$SbCl_3$. This result suggests that 3,5-cholestadiene is present together with 2,4-cholestadiene owing to the presence of TCA, that is, the presence of a protic acid brings about the migration of 2,4-cholestadiene (18). These findings are consistent with the results that the dimers (II) and (III) are produced in the media having the chracters of the Brønsted and the Lewis acids, respectively, while the dimers

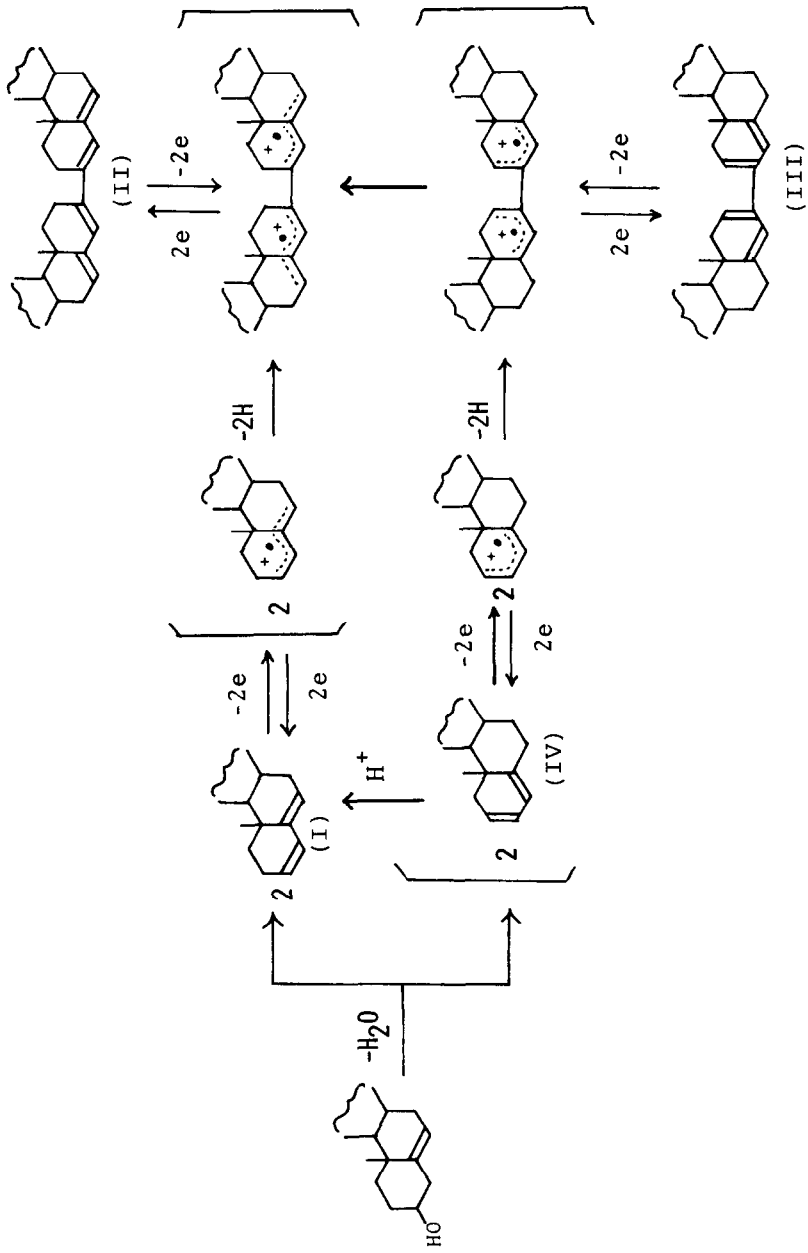

Chart 2

(II) and (III) are simultaneously obtained in the media possessing both characters of the Brønsted and the Lewis acids. Furthermore, it is of interest that the dimer (III) undergoes rearrangement to the dimer (II) in TCA-$SbCl_3$. It was found that the 3,3'-dimer of 2,4-cholestadiene was converted to the 3,3'-dimer of 3,5-cholestadiene as well as the migration of the monomeric cholestadiene in a protic milieu (18).

Experimental

Absorption spectra were measured by Hitachi Recording Spectrophotometer Type EPS-3 in a cell of 10 mm optical length, Infrared (IR) spectra by JASCO IRA-1 Spectrometer, ESR spectra by JEOL JES-ME-1X Spectrometer with manganese monoxide as external standard, and Mass spetcra by JEOL JMS-01S Mass Spectrometer. For TLC, commercially available plates (Merck, DC-Fertigplatten Kieselgel) were used.

Media of the Color Reaction —— A solution of 9 g of trichloroacetic acid liquefied by the addition of 1 ml of water was used as TCA, and a mixture of 20 ml of TCA and 5 g of antimony trichloride as TCA-$SbCl_3$.

Reaction of Cholesterol with TCA-$SbCl_3$ —— To a solution of 5 g of cholesterol in 20 ml of chloroform was added 200 ml of TCA-$SbCl_3$. The solution was stirred for 90 min at a room temperature to give a red colored solution, which was diluted with 10% hydrochloric acid, and extracted with chloroform. The chloroform layer was washed with saturated sodium bicarbonate solution and then water, and dried over sodium sulfate. The evaporation of chloroform left an oily residue, which was taken up in hexane and submitted to column chromatography over silica gel.

Isolation of the Reaction Product —— The first elution with hexane gave an oily substance, which was rechromatographed over alumina with the same solvent, and recrystallization from ethanol-acetone gave analytically pure 3,5-cholestadiene as colorless prisms, (85 mg) m.p. 78-79°. It did not show melting point depression on admixture with the authentic sample prepared by the Mauthner's method (9), and its IR spectrum was identical with that of the authentic sample. Mass spectrum m/e: 368 (M^+).

Further elution with hexane gave crude 3,3'-bis(2,4-cholestadiene). Rechromatography over alumina and recrystallization from chloroform gave colorless needles, (32 mg) m.p. 290-300°. It did not show melting point depression on admixture with the authentic sample prepared by the Owade's method (11), and its IR spectrum was identical with that of the authentic sample. Mass spectrum m/e: 734 (M^+). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 272(4.55), 280(4.61), 292(4.48).

Further elution with hexane gave crude 3,3'-bis(3,5-cholestadiene). Purification was carried out by the same way as above to yield colorless needles, (25 mg) m.p. 244-246°. It did not show melting point depression on admixture with the authentic sample prepared by the Squire's method (10), and its IR spectrum was identical with that of the authentic sample. Mass spectrum m/e: 734 (M^+). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 298(4.70), 312(4.80), 328(4.67).

Further elution with hexane gave crude cholesteryl trichloroacetate. Recrystallization from ligroin gave colorless prisms, (33 mg) m.p. 147-148°. Mass spectrum m/e: 368 (M^+ - 164).

Reaction of 3,5-Cholestadiene with TCA-$SbCl_3$ —— To a solution of 3 g of 3,5-cholestadiene in 20 ml of chloroform was added 100 ml of TCA-$SbCl_3$. The solution was stirred for 90 min at a room temperature and treated by the same procedure as described above. Yield of 3,3'-bis(3,5-cholestadiene) was 79 mg.

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