[Chem. Pharm. Bull. 24(3) 487—491 (1976)]

UDC 574.922.04 : 546.863.131.04

Color Reaction of Cholesterol with the Antimony Trichloride Reagent

Yoshihisa Kurasawa, Atsushi Takada, and Takeo Ueda

School of Pharmaceutical Sciences, Kitasato University[1)]

(Received July 3, 1975)

The reaction of cholesterol with the antimony trichloride reagent was investigated in order to elucidate the coloration mechanism. Two reaction products, 3,5-cholestadiene (I) and 3,3′-bis(2,4-cholestadiene) (II), were isolated from the colored solution. It was assumed that 2,4-cholestadiene (IV) might be produced in the colored solution and IV should change to I during the process of the isolation. I and II were observed to color yellow and then red with the antimony trichloride reagent. It was concluded from the evidence obtained by electron spin resonance study on the colored solution that this coloration might be ascribed to cation-radical. The mechanism of the dimerization of IV to II was examined.

It was reported by Mueller[2)] that various steroidal substances exerted characteristic coloration by reacting with antimony trichloride and that absorption spectra of these colorations would reflect the presence of specific groups on the molecules of steroids, when dissolved in a solution of antimony trichloride and acetyl chloride in chloroform (antimony trichloride reagent[3)]). Mueller claimed that absorption spectra of these colorations should be effectively useful to follow a process of structural changes during the synthesis and decomposition of steroids. He, however, did not isolate any reaction product associated with the coloration, and did not offer any mechanism of the coloration.

As the color reaction using the antimony trichloride reagent, the Carr-Price reaction of vitamin A[4)] and reaction of conjugated polyene compounds[5)] with the antimony trichloride reagent have been known and the mechanisms of the both colorations were ascribed to corresponding cations, which were produced on the course of the reactions. In the color reaction of cholesterol using various Brønsted or Lewis acids, the mechanisms of the coloration have been also explained on the basis of cation formation,[6)] while little attention has been paid to the free radical mechanism. On the other hand, Adams[7)] suggested that the Friedel-Crafts alkalation catalized by aluminium chloride as one kind of Lewis acid, which has been explained on the basis of an ionic mechanism, might proceed through a radical formation from the data of electron spin resonance (ESR) measurements. Since antimony trichloride itself belongs to a Lewis acid, as well as aluminium chloride, the possibility of a free radical mechanism may be anticipated in the color reaction of Mueller. Therefore, it was of interest for the authors to elucidate the mechanism of the Mueller's coloration by using cholesterol as a typical steroidal compound.

By the reaction of cholesterol with the antimony trichloride reagent, the reaction mixture was observed to color yellow in the beginning and then red, and it showed the absorption maxima at 420 and 500 nm as shown in Fig. 1. The colored solution was washed with diluted hydro-

1) Location: *Shirokane, Minato-ku, Tokyo, 108, Japan.*
2) A. Mueller, *J. Am. Chem. Soc.*, **71**, 924 (1949).
3) The solvent and reagent were prepared according to D.T. Ewing, G.V. Kingsley, R.A. Brown, and A.D. Eminett. *Ind. Eng. Chem. Anal. Ed.*, **15**, 301 (1943).
4) P.E. Blatz and A. Estrada, *Anal.* Chem., **44**, 570 (1973).
5) T.L. Jacobs and W.P. Tuttle, Jr., *J. Am. Chem. Soc.*, **71**, 1313 (1949).
6) R. Dulou, J. Chopin, and Y. Raoul, *Bull. Soc. Chim. France*, **1951**, 616; J. Chopin, *ibid.*, **1956**, 258.
7) J.Q. Adams and S.W. Nicksic, *J. Am. Chem. Soc.*, **84**, 4355 (1962).

chloric acid and then water, and extracted with chloroform. The extract was purified by column chromatography on silica gel and two components, 3,5-cholestadiene (I) (91%) and 3,3′-bis(2,4-cholestadiene) (II) (4.1%), were obtained as the reaction products besides a resinous substance. Both of I and II exhibited a yellow coloration initially on dissolving them in the antimony trichloride reagent, and the color changed to red with the passage of time. The absorption maxima of the red solution of I were observed at 362, 420 and 500 nm, and those of the red solution of II at 420 and 500 nm. Therein, the absorption maxima and the spectral pattern of the colored solution of II were found to be identical with those of the colored solution from cholesterol, whereas the spectral pattern of I seemed to differ from that of cholesterol, as shown in Fig. 2 and 3. These results suggested that II was closely related to the colored substance produced by the reaction of cholesterol with the antimony trichloride reagent, but I was not so.

To ascertain this assumption, the colored solution of I with the antimony trichloride reagent was treated by the similar procedure to that described above, and 3,3′-bis(3,5-cholestadiene) (III) was isolated as the reaction product in 2.7% yield, while II was not obtained. From these results together with the fact that III was not obtained from the colored solution

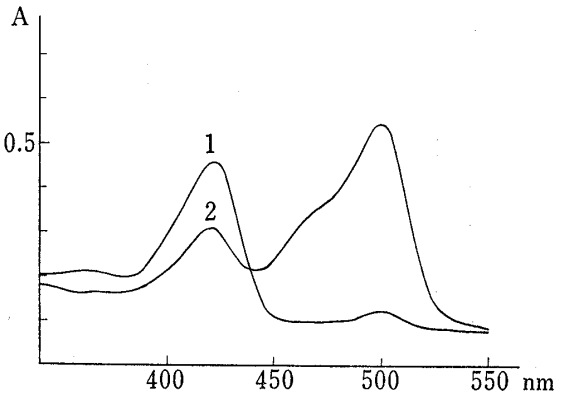

Fig. 1. Absorption Spectra of the Color developed by Cholesterol with the Antimony Trichloride Reagent

A solution of 0.160 mg of cholesterol in 10 ml of antimony trichloride reagent was kept for a few periods. Spectrum 1 was recorded at 24 min and spectrum 2 at 171 min.

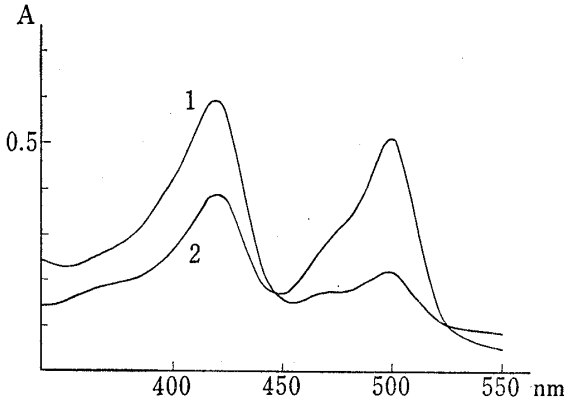

Fig. 2. Absorption Spectra of the Color developed by 3,3′-Bis(2,4-cholestadiene) with the Antimony Trichloride Reagent

A solution of 0.270 mg of 3,3′-bis(2,4-cholestadiene) in 10 ml of antimony trichloride reagent was kept for a few periods. Spectrum 1 was recorded at 60 min and spectrum 2 at 108 min.

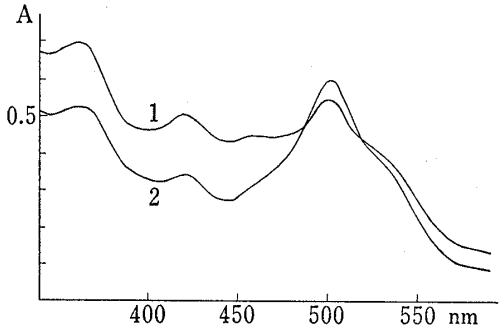

Fig. 3. Absorption Spectra of the Color developed by 3,5-Cholestadiene with the Antimony Trichloride Reagent

A solution of 0.730 mg of 3,5-cholestadiene in 10 ml of antimony trichloride reagent was kept for a few periods. Spectrum 1 was recorded at 30 min and spectrum 2 at 90 min.

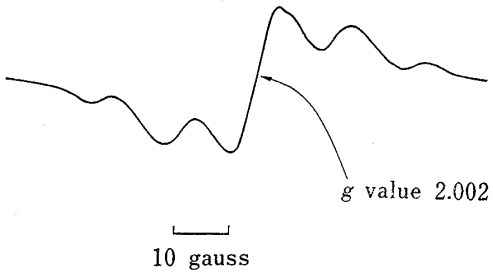

Fig. 4. ESR Spectrum in the Colored Solution of 3,5-Cholestadiene with Antimony Trichloride in Chloroform

of cholesterol with the antimony trichloride reagent, it may be assumed that I was not a direct reaction product in the coloration of cholesterol, and that the other direct reaction product changed to I. Because Stavely[8] had reported that 2,4-cholestadiene (IV) easily migrated to I in an acidic medium. Taking this finding into consideration, it may be concluded in this reaction that cholesterol changed to IV at the first step, following by the dimerization to II in the similar way to that of the formation of III from I, and that unstable IV converted to stable I during the treatment with diluted hydrochloric acid in the isolation process.

As described above, it may be said that IV and II were closely related to the colored substances formed in the Mueller's coloration of cholesterol. In order to elucidate this point of mechanism, the ESR spectra of the colored solutions from cholesterol, I and II were surveyed respectively. As the result obtained, the ESR spectra of all of the colored solutions showed the signals near 3400 gauss, and their *g*-values are shown in Table I. Any of hyperfine structure in all of the ESR spectra could not be observed using the antimony trichloride reagent. However, there was found a hyperfine structure in the reaction of I with a reagent of antimony trichloride in chloroform, as shown in Fig. 4. At the present stage, the authors can not explain about the influence coming from the difference between the antimony trichloride reagent and a reagent of antimony trichloride in chloroform (without acetyl chloride) on the ESR spectra. It may be the most reasonable to conclude from these results that the Mueller's coloration of cholesterol may be ascrided to cation-radical produced from IV and II in the antimony trichloride reagent. Many investigators have claimed as for the coloration of steroids having a conjugated polyene system with various Brønsted or Lewis acids that the corresponding cation formed might contribute to the coloration. Actually, it was found by the authors that the both of ESR spectra of a Liebermann-Burchard reaction mixture and a colored solution of cholesterol in concentrated trichloroacetic acid solution showed weak signals which was negligible. In the Carr-Price reaction, the coloration of vitamin A with the antimony trichloride reagent has been also assumed due to the corresponding cation. However, the above results suggested that the free radical formation also in the Carr-Price reaction mixture may be expected with a considerable possibility. To confirm this assumption, an attempt was made to measure the ESR spectrum of the Carr-Price reaction mixture, a signal of free radical at *g*-value 2.002 was observed, indicating that the coloration of vitamin A with the antimony trichloride reagent might also be ascribed to cation-radical. Further ESR studies of the Carr-Price reaction and its related one are in progress and the results will be reported in the near future.

As pointed out above, it was found that II might be produced from IV by its dimerization in the colored solution. In order to decide whether this dimerization has a radical nature, the reaction product of the coloration of I, in lieu of IV, in the antimony trichloride reagent with and without diphenylpicrylhydrazyl (DPPH) as a sweeping agent were pursued, since IV was unstable and hardly available under the present reaction condition. From the colored solution containing DPPH, any of dimeric compound such as III was not obtained, and besides a resinous substance two other products, picramide and 4-chloro-diphenylamine, were isolated

Table I. Respective *g* Values in the Reaction of the Steroids with the Antimony Trichloride Reagent

Compound	*g* Value
Cholesterol	2.003
3,5-Cholestadiene	2.002
3,3′-Bis(2,4-cholestadiene)	2.003
3,3′-Bis(3,5-cholestadiene)	2.003

8) H.E. Stavely and W. Bergmann, *J. Org. Chem.*, **1**, 575 (1937).

from the reaction mixture, while the colored solution of I without DPPH gave III as described above. Among these compounds, picramide and 4-chlorodiphenylamine were also found to be obtained in the reaction of DPPH with the antimony trichloride reagent. On the basis of these findings, it may be said that the dimerization of I to III proceed radically and DPPH block this dimerization.

As described above, it was found that IV was the main product of the Mueller's reaction, further IV dimerized to II radically, and the coloration was ascribed to cation-radicals produced from IV and II. The overall process are shown in Chart 1.

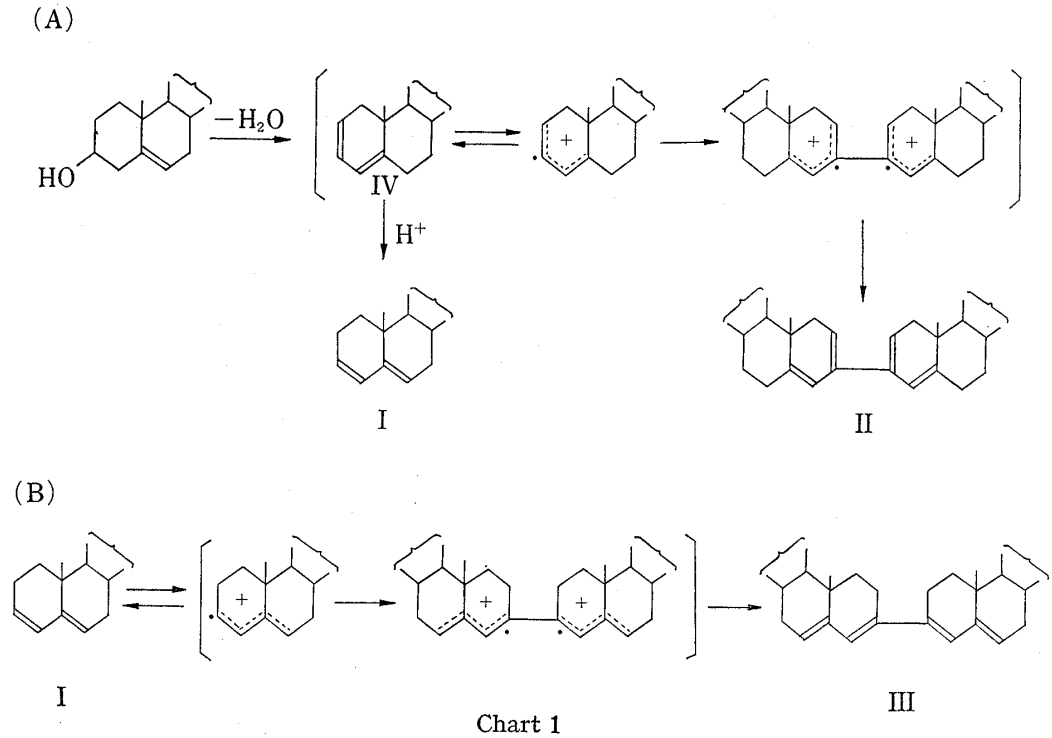

Chart 1

Experimental[9]

Color Reaction of Cholesterol with the Antimony Trichloride Reagent——A solution of 15 g of cholesterol in 200 ml of antimony trichloride reagent was stirred for 90 minutes at a room temperature. A red colored solution was washed with 10% hydrochloric acid, extracted with chloroform and the extract was dried over Na_2SO_4.

Isolation of 3,3′-Bis(2,4-cholestadiene)——The above extract was evaporated *in vacuo*. A residue obtained was dissolved in a small amount of hexane with heating on a boiling water bath and then cooled in an ice bath. Insoluble crystalline product was collected by suction, purified by repeated chromatography on alumina with chloroform and then recrystallization from petroleum ether to yield colorless needles, mp 290—300°. Yield, 615 mg (4.1%). It showed no melting point depression on admixture with the authentic sample prepared by the Owade's method.[10] Mass Spectrum *m/e*: 734 (M^+). Ultraviolet (UV) $\lambda_{max}^{CHCl_3}$ nm ($\log\varepsilon$): 272 (4.55), 280 (4.61), 292 (4.48). *Anal.* Calcd. for $C_{54}H_{86}$: C, 88.21; H, 11.79. Found: C, 88.01; H, 11.86.

Isolation of 3,5-Cholestadiene——The above filtrate was evaporated and then chromatographed on alumina with hexane to give colorless crystals. Recrystallization from acetone–ethanol gave colorless prisms, mp 78—79°. Yield, 13.5 g (91%). It showed no melting point depression on admixture with the authentic sample prepared by the Mauthner's method.[11] Mass Spectrum *m/e*: 368 (M^+). UV λ_{max}^{hexane} nm (log ε): 230 (4.39), 235 (4.42), 244 (4.24). *Anal.* Calcd. for $C_{27}H_{44}$: C, 87.96; H, 12.04. Found: C, 87.92; H, 11.84.

9) Absorption spectra were measured by Hitachi Recording Spectrophotometer Type EPA-3 in a cell of 10 mm optical length, infrared (IR) spectra by JASCO IRA-1 Spectrophotometer, ESR spectra by JEOL JES-ME-1X Spectrometer with manganese monoxide as external standard, and mass spectra by JEOL JMS-OlS Mass Spectrometer.

10) J.L. Owade and A.E. Sobel, *J. Am. Chem. Soc.*, **73**, 4223 (1951).

11) J. Mauthner and W. Suida, *Monatsh.*, **17**, 29 (1896).

Reaction of 3,5-Cholestadiene with the Antimony Trichloride Reagent——A solution of 3 g of 3,5-cholestadiene in 100 ml of antimony trichloride reagent was stirred for 90 minutes at a room temperature. The colored solution was treated as described above.

Isolation of 3,3′-Bis(3,5-cholestadiene)——Crude 3,3′-bis(3,5-cholestadiene) was obtained by dissolving the reaction product in a small amount of hexane and then cooling in an ice bath. Column chromatography on alumina with chloroform and then recrystallization from chloroform gave colorless needles, mp 244—246°. Yield, 80 mg (2.7%). It showed no melting point depression on admixture with the authentic sample prepared by the Squire's method.[12] Mass Spectrum *m/e*: 734 (M^+). UV $\lambda_{max}^{CHCl_3}$ nm (logε): 298 (4.70), 312 (4.80), 328 (4.67). *Anal.* Calcd. for $C_{54}H_{86}$: C, 88.21; H, 11.79. Found: C, 87.99; H, 11.82.

Reaction of 3,5-Cholestadiene with the Antimony Trichloride Reagent in the Presence of DPPH——A solution of 1 g of 3,5-cholestadiene and 1 g of DPPH in 100 ml of antimony trichloride reagent was treated by the same procedure as described above.

Isolation of Picramide——The above extract was evaporated, and the residue was dissolved in a small amount of chloroform in order to separate the insoluble crystalline product. A yellowish substance precipitated was filtrated, and recrystallization from acetic acid and then acetic anhydride gave prisms, mp 187—188°. Yield, 310 mg (54.7%). It showed no melting point depression on admixture with the authentic sample prepared by the method of Witt.[13] Mass Spectrum *m/e*: 228 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3460, 3340 (NH_2), 1530, 1355 (NO_2), 1280 (C–N, aromatic amine). *Anal.* Calcd. for $C_6H_4O_6N_4$: C, 31.59; H, 1.77; N, 24.56. Found: C, 31.30; H, 1.69; N, 24.29.

Isolation of 4-Chloro-diphenylamine——The above filtrate was evaporated, and the residue was dissolved in a small amount of hexane and submitted to column chromatography on silica gel with hexane. After the elution of unreacted 3,5-cholestadiene, crude 4-chloro-diphenylamine was obtained. Recrystallization from ethanol–water gave colorless needles, mp 70—71°. Yield, 70 mg (13.6%). It showed no melting point depression on admixture with the authentic sample prepared from 4-chloro-acetanilide and bromobenzene according to the method of Weston.[14] Mass Spectrum *m/e*: 203 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3420, 1590 (NH), 1315 (C–N). *Anal.* Calcd. for $C_{12}H_{10}NCl$: C, 70.77; H, 4.95; N, 6.88. Found: C, 70.63; H, 4.90; N, 6.78.

Reaction of DPPH with the Antimony Trichloride Reagent——A solution of 1 g of DPPH in 100 ml of antimony trichloride reagent was stirred for 90 minutes at a room temperature and treated by the similar manner to that as described above. The chloroform extract was evaporated *in vacuo*. By treating the residue as described above, 360 mg of picramide and 140 mg of 4-chloro-diphenylamine were obtained.

Acknowledgement The authors are indebted to Mr. H. Nagao for his technical assistances.

12) E.N. Squire, *J. Am. Chem. Soc.*, **73**, 2586 (1951).
13) O.N. Witt and E.W. Witte, *Ber.*, **41**, 3090 (1908).
14) P.E. Weston and H. Adkins, *J. Am. Chem. Soc.*, **50**, 859 (1928).