[Chem. Pharm. Bull. 24(6)1268—1272(1976)]

UDC 547.922.04.08 : 546.863.131.04

Color Reaction of Estrone with the Antimony Trichloride Reagent

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(Received September 30, 1975)

The color reaction of estrone with the antimony trichloride reagent was investigated in order to clarify the reaction mechanism. Two reaction products, 3′-methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydrophenanthrene (I) and estrone acetate (II), were isolated from the colored reaction mixture. It was found that acetyl chloride is indispensable for the color reaction, and that the respective steroids are colored by the formation of the cation-radicals. It was assumed that the cation-radical of the substance I should be responsible for the coloration. The reaction mechanism for the formation of the substance I was inferred, and the relationship between the absorption maxima observed in the absorption spectra and the cation-radicals was discussed.

In the previous paper,[2] the authors reported the color reaction of cholesterol with the antimony trichloride reagent provided by Mueller,[3] and clarified that the reaction products were colored yellow and then red by the formation of the cation-radicals in this color reaction, and that the dimerization of the monomers such as 3,5-cholestadiene took place radically.

In the continuation of this type of reaction, the color reaction of estrone, one of the steroids possessing the phenolic structure in the ring A, with the antimony trichloride reagent was studied to elucidate the reaction mechanism.

A solution of estrone in the antimony trichloride reagent was stirred at a room temperature. The solution was observed to color yellow initially and then yellowish red, and the red coloration increased in intensity ultimately. The absorption spectra of the colored solution are shown in Fig. 1, and the absorption maxima are observed at 470 and 510 nm. It was found in this coloration that, after 24 hours, the absorption maximum at 470 nm disappeared, and only the absorption maximum at 510 nm remained. The red colored reaction mixture after the interval of 24 hours was washed with 10% hydrochloric acid and then water, and the reaction product was extracted with chloroform and dried over sodium sulfate. After the evaporation of chloroform, the oily residue obtained was submitted to column chromatography on silica gel to give two substances I and II.

Substance I was colorless crystals of mp 95—96°. The data of the elementary analysis and the mass spectrum (M^+ 292) revealed the formula $C_{20}H_{20}O_2$. The substance I, therefore, was presumed as 3′-methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydrophenanthrene, shown in Chart 1, from the inspection of the spectral data as follows. In the infrared (IR) spectrum, the absorption band of carbonyl group due to acetate was observed at 1760 cm^{-1}, but not the absorption bands of 3-hydroxyl and 17-carbonyl groups shown in the IR spectrum of estrone. In the nuclear magnetic resonance (NMR) spectrum, the substance I showed a doublet signal (J=7 Hz, 1.19 ppm) for secondary 3′-methyl protons, a singlet signal (2.32 ppm) for methyl protons of 7-acetoxy group, and signals (7.87—6.67 ppm) for five aromatic protons, but no signal for olefinic proton was observed. The ultraviolet (UV) spectrum exhibited the absorption maximum at 278 nm (solvent, isooctane or methylene chloride) as shown in Fig. 2. When the rings A and C are the aromatic ring, the UV absorption maximum is 282 nm (solvent, isooctane), and the chemical shift of a doublet signal (J=7 Hz) for 3′-methyl protons is 1.30

1) Location: *Shirokane, Minato-ku, Tokyo, 108, Japan.*
2) Y. Kurasawa, A. Takada, and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **24**, 487 (1976).
3) A. Mueller, *J. Am. Chem. Soc.*, **71**, 924 (1949).

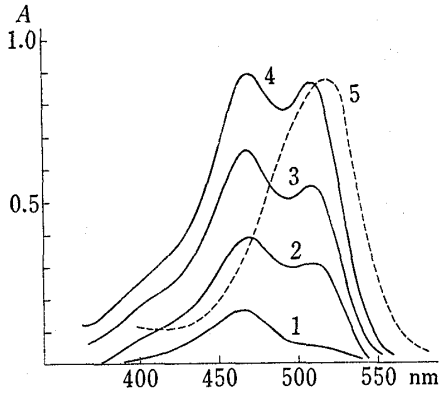


Fig. 1. Absorption Spectra of Estrone with the Antimony Trichloride Reagent

A solution of estrone in the antimony trichloride reagent was allowed to stand for several periods at a room temperature. Spectra 1, 2, 3, 4, and 5 were recorded at 0.5, 3, 4, 6, and 24 hr respectively. Concentration in the spectra 1—4 is 0.695 mg/ml, and that in the spectrum 5 is 0.348 mg/ml.

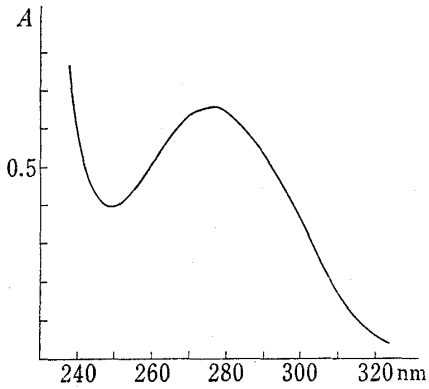
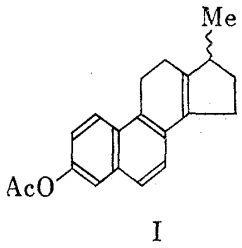


Fig. 2. UV Spectrum of 3'-Methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydrophenanthrene

1.72 mg of 3'-methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydro-phenanthrene was dissolved in 100 ml of methylene chloride.

ppm.[4] Therefore, it was assumed that the A and B rings should exist as aromatic, but not the C ring. As for the configuration of 3'-methyl group, Kimura, *et al.*[4] have shown to be isomeric mixtures. The configuration of the 3'-methyl group in the present investigation was assumed to be same as that in the result of Kimura.



I

Chart 1

Substance II was colorless needles of mp 126—127°. From the spectral data, the substance II was identified as estrone acetate.

The substance I was observed to color yellowish red instantaneously and red several minutes later, on shaking with the antimony trichloride reagent. The absorption spectra of the colored solution are shown in Fig. 3, and the absorption maxima are observed at 470 nm (spectrum 1) and 510 nm (spectra 2, 3, and 4). However, the substance II showed no coloration immediately, whereas the gradual coloration with the antimony trichloride reagent similar to that of estrone was observed. The absorption spectra of the colored solution are shown in Fig. 4, and the absorption maxima are observed at 470 and 510 nm. These results suggest that the substance I should be responsible for the coloration, and estrone should change into the substance II and then into the substance I in the antimony trichloride reagent.

Since the steroidal coloration with the antimony trichloride reagent was found to relate to the formation of the cation-radical in the previous paper,[2] the electron spin resonance (ESR) measurement was carried out in order to confirm the formation of the free radical. The results are shown in Table I. The free radicals were detected in the red colored solution of the respective steroids. In the respective ESR spectra, any of the hyperfine structure was not observed. It has been reported by many investigators[5] that the ESR spectra of the phenoxy radicals were measured in the reaction of phenol derivatives with metal ions. In the present investigation, however, it is not evident whether the free radicals of the steroids measured are the phenoxy radicals or the cation-radicals. It was found that the reaction

4) M. Kimura, M. Kawata, K. Akiyama, K. Harita, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **21**, 1741 (1973).

5) T.J. Stone and W.A. Waters, *Proc. Chem. Soc.*, **1962**, 253; L.M. Stock and J. Suzuki, *Proc. Chem. Soc.*, **1962**, 136.

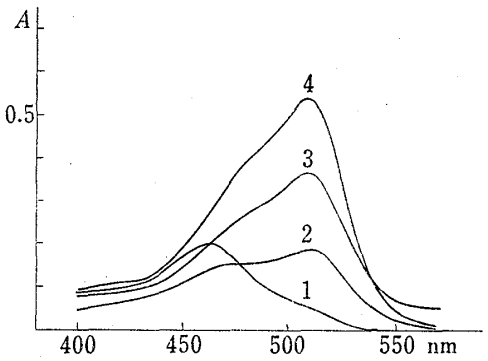


Fig. 3. Absorption Spectra of 3'-Methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydrophenanthrene with the Antimony Trichloride Reagent

A solution of 3'-methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydrophenanthrene in the antimony trichloride reagent was allowed to stand for several periods at a room temperature. Spectra 1, 2, 3, and 4 were recorded at 0, 0.5, 2, and 4 hr respectively. Concentration of the solution is 0.529 mg/ml.

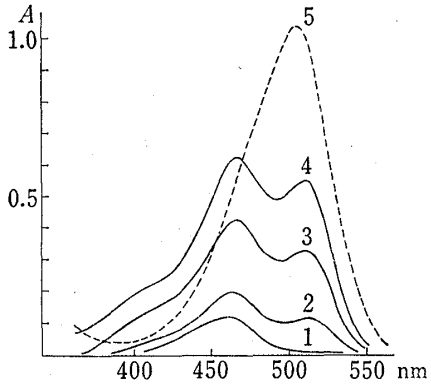


Fig. 4. Absorption Spectra of Estron Acetate with the Antimony Trichloride Reagent

A solution of estrone acetate in the antimony trichloride reagent was allowed to stand for several periods at a room temperature. Spectra 1, 2, 3, 4, and 5 were recorded at 0.5, 2, 4, 6, and 24 hr respectively. Concentration in the spectra 1—4 is 0.814 mg/ml, and that in the spectrum 5 is 0.271 mg/ml.

products were isolated as acetates and the acetates were colored by the formation of the free radical, and that the substance I was colored red on shaking with the chloroform solution of antimony trichloride (without acetyl chloride). Therefore, it may be said that the cation-radical, but not the phenoxy radical, should be produced in the antimony trichloride reagent.

It has been reported by Baughan, *et al.*[6] that the cation-radicals of the aromatic hydrocarbons are not formed with antimony trichloride, when completely free from oxygen or antimony pentachloride. Because antimony trichloride itself is not an oxidizing agent, it was postulated that the dehydrogenation in the B ring might be brought about by oxygen. In order to confirm this postulation, the reaction of estrone with the antimony trichloride reagent was carried out, conducting oxygen into the reaction mixture with stirring. As the result, the red coloration was found to be observed earlier than that under the condition without the introduction of oxygen as shown in Fig. 1 and 4. The absorption spectra of the colored solution are shown in Fig. 5. These findings indicate that the formation of the substance I should be promoted by oxygen.

It was also presumable that 17-carbonyl group should be converted to the hydroxyl group

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

Compound	g Value
Estrone	2.004
Substance I	2.004
Substance II	2.004

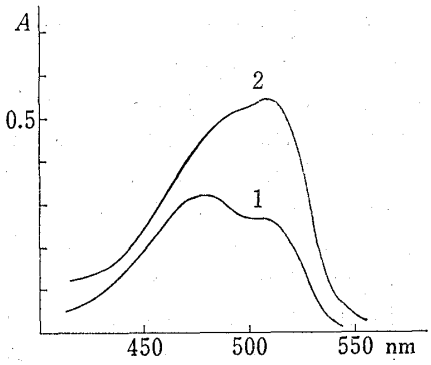


Fig. 5. Absorption Spectra of Estrone with the Antimony Trichloride Reagent

To a solution of estrone in the antimony trichloride reagent was conducted oxygen with stirring at a room temperature. Spectra 1 and 2 were recorded at 1 and 3 hr respectively. Concentration of the solution is 1.01 mg/ml.

6) E.C. Baughan, T.P. Jones, and L.G. Stoodley, *Proc. Chem. Soc.*, 274 (1963).

concomitant with the dehydrogenation in the B ring, although the mechanism and the configuration of the resulting hydroxyl group were not made clear. This type of reaction has been reported by Kimura, *et al.*,[7] who investigated the reaction of estrone or estradiol with 78% sulfuric acid. Since the protonation could not occur in the antimony trichloride reagent, it was inferred that acetyl cation should add to 17-hydroxyl group, and then the migration of 18-methyl group to 17-position should come about accompanied by the elimination of acetic acid. This inference seems to be supported by the fact that, without acetyl chloride in the antimony trichloride reagent, the red coloration was not developed, while the reaction mixture was colored yellow. Based on the above findings, it may be postulated that the reaction mechanism proceeded as shown in Chart 2.

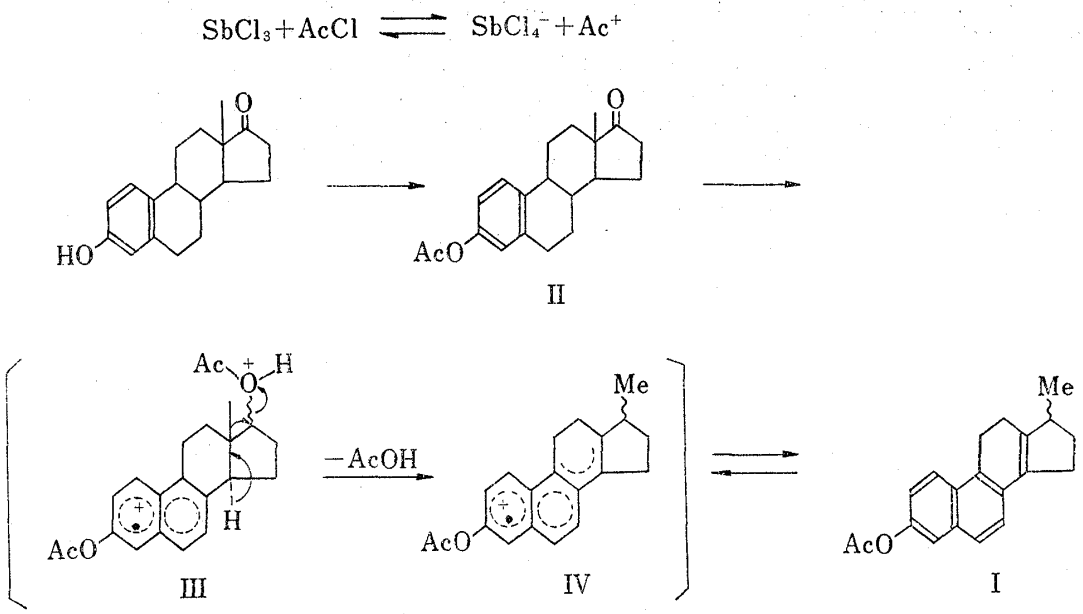


Chart 2

As described above in Fig. 1 and 4, at the beginning of the reaction the absorption maximum was observed only at 470 nm, then the absorption maximum at 510 nm increased together with that at 470 nm, and after 24 hours only the absorption maximum at 510 nm was observed. The shift of the absorption maximum from 470 to 510 nm should be attributed to the increase of a conjugated double bond. Such an instance has been demonstrated by Kimura, *et al.*[8] in the reaction of estradiol with 78% sulfuric acid. Their case is the shift from 465 to 515 nm. From these findings, it was assumed that the absorption maximum at 470 nm should be ascribed to the aromatic cation-radical of an intermediate such as III in Chart 2, and the absorption maximum at 510 nm to the cation-radical of the substance I such as IV in Chart 2.

Experimental[9]

Reaction of Estrone with the Antimony Trichloride Reagent——A solution of 3 g of estrone in 200 ml of the antimony trichloride reagent was stirred for 24 hours at a room temperature. The solution was colored

7) M. Kimura, K. Akiyama, K. Harita, T. Miura, and M. Kawata, *Tetrahedron Letters*, **1970**, 377.
8) M. Kimura, K. Akiyama, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **20**, 2511 (1972); *idem*, *ibid.*, **22**, 643 (1974).
9) Absorption spectra were measured by Hitachi Recording Spectrophotometer Type EPS-3 in a cell of 10 mm optical length, IR spectra by JASCO IRA-1 Spectrophotometer, ESR spectra by JEOL JES-ME-1X Spectrometer with manganese monoxide as external standard, MS by JEOL JMS-OlS Mass Spectrometer, and NMR spectra by Varian T-60 Spectrometer at 60 MHz with tetramethylsilane as internal standard.

yellow initially, and colored yellowish red about 3 hours later. The red coloration gradually increased in intensity.

To the red colored solution was added 100 ml of acetone with stirring. The solution was washed with 10% hydrochloric acid three times and then with water. The solution was dried over Na_2SO_4, and evaporated *in vacuo* to leave an oily residue. The oily residue was dissolved in a small amount of chloroform to submit to column chromatography on silica gel eluting with chloroform. The first yellow nonfluorescent fraction was collected, and chloroform was evaporated *in vacuo* to give an oily substance. The oily substance obtained was rechromatographed on alumina eluting with chloroform. This purified product was treated with methanol to crystallize. Recrystallization from methanol yielded colorless crystals. Further recrystallization from methanol-water gave analytically pure 3′-methyl-7-acetoxy-1,2-cyclopenteno-3,4-dihydrophenanthrene (I) as colorless crystalline powder, mp 95—96°. Yield, 53 mg. Mass Spectrum *m/e*: 292 (M^+). IR ν_{max}^{KBr} cm^{-1}: 2960, 2880 (CH_2), 1760, 1245 (acetyl C=O), 1608, 1500 (aromatic ring C=C). NMR ppm ($CDCl_3$): 7.87—6.67 (5H, aromatic protons), 2.32 (3H, s, acetyl CH_3), 1.19 (3H, d, 3′-CH_3, $J=7$ Hz). UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 278 (4.0). *Anal.* Calcd. for $C_{20}H_{20}O_2$: C, 82.16; H, 6.90; O, 10.94. Found: C, 81.89; H, 6.98.

The original silica gel column was eluted with chloroform successively. The chloroform solution was evaporated *in vacuo* to give another colorless crystals. Recrystallization from ethanol gave analytically pure estrone acetate (II) as colorless needles, mp 126—127°. Yield, 595 mg. Mass Spectrum *m/e*: 312 (M^+). IR ν_{max}^{KBr} cm^{-1}: 2950, 2880 (CH_2), 1765, 1210 (acetyl C=O), 1740 (17–C=O), 1605, 1500 (aromatic ring C=C). *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74; O, 15.36. Found: C, 77.07; H, 7.74.

Acknowledgment The authors thank Miss Akiko Furuuchi for her technical assistance.