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Color and Fluorescence Reaction Mechanism of Progesterone with Sulfuric Acid[1]

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The ^{1}H- and ^{13}C-nuclear magnetic resonance spectra of progesterone (VII) in 97% H_2SO_4 indicated the formation of a dication (VIII), which was transformed to another dication χ-301 (X) consisting of a hydroxyalkenyl cation moiety in ring A and a cyclopentenyl one in ring D, when the acid solution was heated at 80 °C for 2 h. At a reduced acid-strength of the reaction system, X was converted to the hydroxyalkatrienyl cation χ-482 (XIV) through an acid-base equilibrium on the one hand, and to the hydroxyalkatetraenyl cation χ-600 (XV) by an oxidative process on the other. The structures of these cations were confirmed by isolating the corresponding conjugate bases, 17ξ-isopropyl-18-nor-13ξ-androsta-4,6,8(14)-trien-3-one (XII) and 17-isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one (XIII), from the colored solutions. The pK_a values of the cations XIV and XV were -4.6 and -3.8, respectively. On the basis of these results, a mechanism is proposed for the title reaction.

Keywords——progesterone; sulfuric acid; ^{1}H-NMR; ^{13}C-NMR; 17ξ-isopropyl-18-nor-13ξ-androsta-4,6,8(14)-trien-3-one; 17-isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one; cyclopentenyl cation; hydroxyalkatrienyl cation; hydroxyalkatetraenyl cation; pK_a of carbocation

Many of the color and fluorescence reactions of steroidal compounds with strong acids are well known and have been utilized for the determination of these compounds in the body fluids and in pharmaceutical preparations. However, little is known on the mechanisms of these reactions. During the course of this series of studies, the mechanisms of the color and fluorescence reactions of steroidal estrogen and androgen with strong acid have been investigated.[1b,2-7] It was clarified that the chromo- and fluorophores thus formed are steroidal carbocations. Protonation, dehydration, rearrangement, and oxidation processes participate in the similar reaction of testosterone (I) and two chromo- and fluorophores, hydroxyalkatrienyl cation IV ($\lambda_{max}=484$ nm, $\lambda_{em}=530$ nm)[5,6] and hydroxyalkatetraenyl cation V ($\lambda_{max}=600$ nm, $\lambda_{em}=615$ nm),[1b] are produced through the formation of the dications, II ($\lambda_{max}=292$ nm) and III ($\lambda_{max}=300$ nm).[4,7] These results may offer a clue for elucidating the color and fluorescence reaction mechanisms of the similar reactions of 4-en-3-one steroids such as progesterone (VII)[8] and corticosteroid.[9] In fact, the chromo- and fluorophores produced in the reactions of VII and corticoid with sulfuric acid showed absorption and emission maxima similar to those of IV and V. Presumably, these products have a common structural moiety. We discuss, in this paper, the chemical structures and the formation mechanism of the chromo- and fluorophores formed in the reaction of VII with sulfuric acid.

Results and Discussion

An absorption maximum, which has an apparent molecular extinction coefficient (ε') of 17000 and is rather stable at room temperature, appeared rapidly at 291 nm when pro-

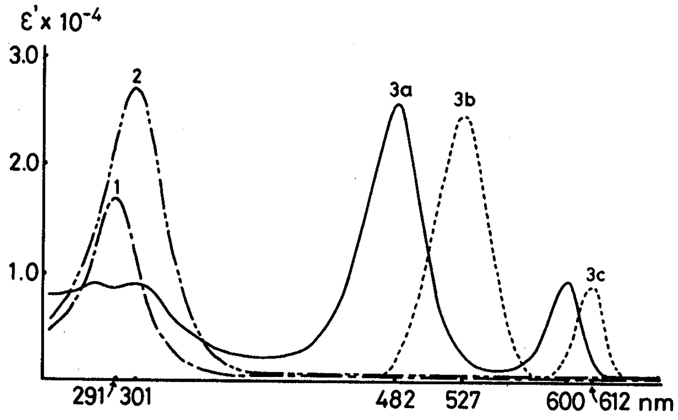

Fig. 1. Absorption and Fluorescence Spectra of Progesterone (VII) in the Sulfuric Acid–Ethanol System

1: VII (50 μg) was dissolved in 97% H_2SO_4 (4 ml). Absorption spectrum. 2: The same solution as in 1 was heated at 80 °C for 2 h. Absorption spectrum. 3: The solution described in 2 (2 ml) was poured into ethanol (4 ml). 3a: Absorption spectrum. 3b and 3c: Fluorescence spectra with excitation at 467 and 593 nm, respectively.

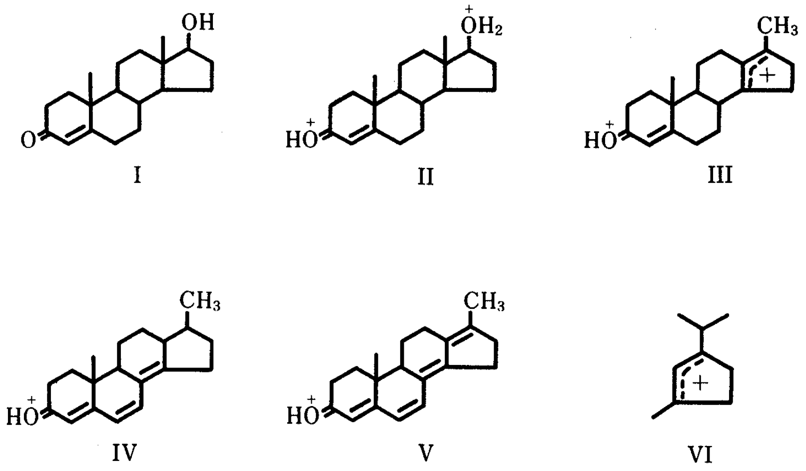

Chart 1

gesterone (VII) was dissolved in 97% sulfuric acid (Fig. 1). No absorption maximum was observed in the visible region on dilution of the reaction mixture with ethanol or water. When the solution of VII in sulfuric acid was heated at 80 °C, the maximum at 291 nm was slightly red-shifted with increased intensity and after 2 h a chemical species (abbreviated as χ-301) was formed, which gave a maximum at 301 nm ($\varepsilon' = 26800$). Dilution of the reaction mixture with ethanol or water reduced χ-301 and produced a new species (abbreviated as χ-482) having a maximum at 482 nm ($\lambda_{em} = 527$ nm) and a species (abbreviated as χ-600) having a maximum at 600 nm ($\lambda_{em} = 612$ nm). This may indicate that χ-301 is a common intermediate to χ-482 and χ-600.

An α,β-unsaturated ketone can be protonated to give the hydroxyalkenyl cation showing a maximum at about 290 nm.[10] In the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of VII dissolved in 97% sulfuric acid at room temperature (Fig. 2a), signals due to C(4)–H, C(20)–CH_3, C(10)–CH_3, and C(13)–CH_3 were easily assigned by comparison with the spectra of II and VII (Table I). The species showing an absorption maximum at 291 nm was thus indicated to be the doubly protonated progesterone (VIII). In fact, VII was recovered almost quantitatively from the sample solution after the ^{1}H-NMR measurement.

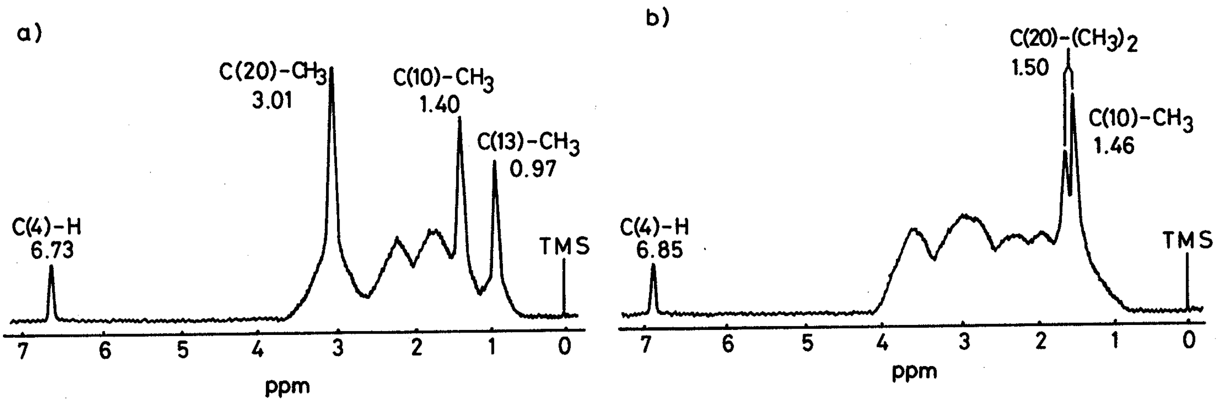

Fig. 2. ^{1}H-NMR Spectra of Progesterone (VII) in 97% Sulfuric Acid

a): VII (40 mg) was dissolved in 0.5 ml of 97% H_2SO_4.
b): The same solution of VII was heated at 80 °C for 2 h.

Table I. ^{1}H-NMR Data[a] for Unsaturated Ketosteroids in $CDCl_3$ or H_2SO_4

	VII[b]	XII[b]	XIII[b]	II[d]	III[c]
C(4)–H	5.74	5.73	5.73	6.82	6.88
C(6)–H		6.02 (d, $J=9$ Hz)	6.01 (d, $J=9$ Hz)		
C(7)–H		6.61 (d, $J=9$ Hz)	6.63 (d, $J=9$ Hz)		
C(10)–CH_3	1.20	0.99	1.03	1.50	1.51
C(13)–CH_3	0.69			1.12	
C(17)–CH_3					3.11
C(20)–CH_3	2.12	0.89 (d, $J=7$ Hz) 0.99 (d, $J=7$ Hz)	1.04 (d, $J=7$ Hz) 1.07 (d, $J=7$ Hz)		

	IV[e]	V[f]	VIII[c]	X[c]	XIV[e]
C(4)–H	6.59	6.02	6.73	6.85	6.54
C(6)–H	6.68 (d, $J=8$ Hz)	6.43 (d, $J=8$ Hz)			6.63 (d, $J=8$ Hz)
C(7)–H	7.74 (d, $J=8$ Hz)	7.60 (d, $J=8$ Hz)			7.59 (d, $J=8$ Hz)
C(10)–CH_3	1.20	1.02	1.40	1.46	1.12
C(13)–CH_3			0.97		
C(17)–CH_3	1.20	2.11			
C(20)–CH_3			3.01	1.50 (d, $J=6$ Hz)	1.07 (d, $J=6$ Hz)

a) ppm from tetramethylsilane as an internal or external standard.
b) In $CDCl_3$. c) In 97% H_2SO_4. d) In 90% H_2SO_4. e) In 60% D_2SO_4. f) In 53% D_2SO_4.

The ^{1}H-NMR spectrum of χ-301 was different from that of VIII. A doublet signal ($J=$ 6 Hz) due to the isopropyl group appeared at 1.50 ppm and the relative area-intensity was increased in the region of about 3.0 to 3.5 ppm (Fig. 2b). Deno *et al.* observed the ^{1}H-NMR spectra of several cyclopentenyl cations and reported that 1-isopropyl-3-methylcyclopentenyl cation (VI) was stable in concentrated sulfuric acid, showing signals at 1.43 (doublet) and 3.54 ppm due to methyl protons of the isopropyl group and methylene protons (C(4)– or C(5)–H) α to the alkenyl cation moiety, respectively.[11a] It was reported that alkenyl cations show maximum absorption at about 300 nm[11b] and that androst-4-en-3-one in 97% sulfuric acid gave a maximum at 295 nm ($\varepsilon=13000$), showing no red-shift.[4] Thus, it may reasonably be considered that χ-301 is the dication X containing a cyclopentenyl cation moiety as well as a hydroxyalkenyl one, and that the red-shift with increased intensity of light absorption cited above is due to formation of the cation moiety in ring D. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of χ-301 in 97% sulfuric acid (Fig. 3) was different from that of VIII (Table II), showing signals (212.3, 121.4, and 206.3 ppm) due to C(3), C(4), and C(5) of the

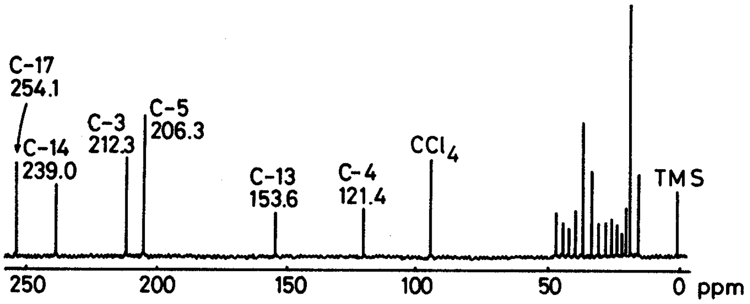

Fig. 3. ^{13}C-NMR Spectrum of χ-301 (X) in 97% Sulfuric Acid

A solution of VII (40 mg) in 97% H_2SO_4 (0.5 ml) was heated at 80 °C for 2 h.

TABLE II. ^{13}C-NMR Data[a] for Unsaturated Ketosteroids in $CDCl_3$ or H_2SO_4

	VII[b]	XII[b]	XIII[b]	III[c]	VIII[c,d]	X[c]
C(3)	199.3	199.3	199.1	212.2	212.8	212.3
C(4)	123.9	122.9 (123.2)[e]	122.3 or 122.8	121.3	120.2	121.4
C(5)	170.8	164.3	164.4	205.8	211.0	206.3
C(6)		124.1 (123.8)[e]	122.3 or 122.8			
C(7)		133.7 (134.9)[e]	134.0			
C(8)		125.3	123.0			
C(13)			134.3	155.2		153.6
C(14)		151.6	152.7 or 154.3	239.1		239.0
C(17)			152.7 or 154.3	245.6		254.1
C(20)	209.1				245.0	

a) ppm from tetramethylsilane as an internal or external standard.

b) In $CDCl_3$. c) In 97% H_2SO_4.

d) These values are in fair agreement with the results of Butler *et al.*[12]

e) The value in parenthesis is that of the other isomer.

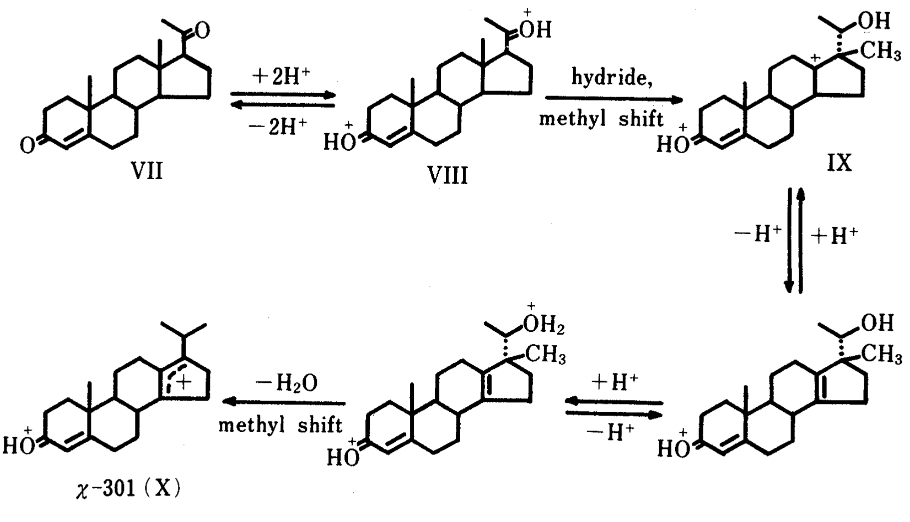

Chart 2. Primary Process of the Color Reaction of Progesterone (VII) with Sulfuric Acid

hydroxyalkenyl cation and signals (153.6, 239.0, and 254.1 ppm) due to C(13), C(14), and C(17) of the cyclopentenyl cation. Five signals, other than the one due to C(17), agreed fairly well in the values of chemical shift with those due to III derived from testosterone (I), as summarized in Table II. These results suggest that χ-301 is the dication (X), which has the hydroxyalkenyl cation moiety at C(3)–C(4)–C(5) as well as the cyclopentenyl one at C(17)–C(13)–C(14). The reaction of progesterone (VII) with 97% sulfuric acid may, therefore, proceed initially as shown in Chart 2. Carbonyl groups of C(3) and C(20) of VII are protonated to give VIII, which is stable at room temperature. Upon heating, hydrogen of C(17) transfers to C(20), followed by rearrangement of the C(13)-methyl group to C(17), giving the unstable cation IX, which is then protonated again, dehydrated, and finally transformed to the stable alkenyl cation χ-301 (X) through a second rearrangement of the methyl group. Thus, the same chromophore moieties (hydroxyalkenyl and cyclopentenyl cations) as those of III formed from testosterone (I) can be derived from VII in concentrated sulfuric acid.

It is known that the stability of III is governed by the acid-strength of the system and the change of III to IV and V occurs with decrease in the strength.[5] Solutions of χ-301 in 97%

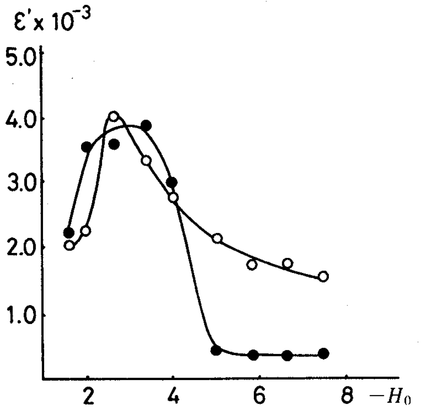

Fig. 4. Effect of Acid-Strength on the Formation of χ-482 and χ-600

A 97% H_2SO_4 solution (50 μl) of X (3 μg/μl) was added to H_2SO_4 (4 ml) of various concentrations, and the absorbance at 482 (○) or 600 nm (●) was measured after 1 h at room temperature.

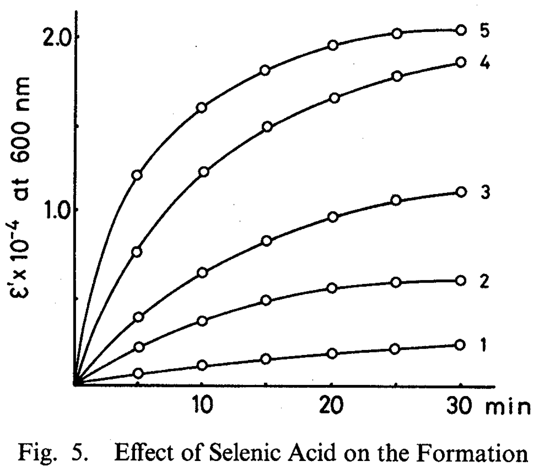

Fig. 5. Effect of Selenic Acid on the Formation of χ-600 (XV)

A 97% H_2SO_4 solution (1 ml) containing VII (1.123 mg) was heated at 80 °C for 2 h and the solution (50 μl) was added to 51% H_2SO_4 (4 ml) containing H_2SeO_4. The final concentrations of H_2SeO_4 were: 1 (0%), 2 (0.0005%), 3 (0.0010%), 4 (0.0030%), and 5 (0.0050%).

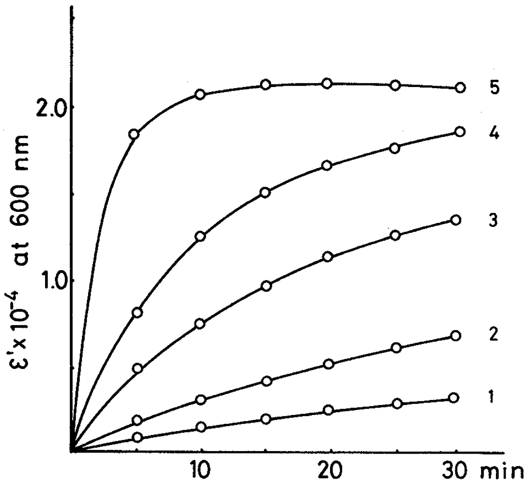

Fig. 6. Effect of Selenic Acid on the Formation of χ-600 (XV) in Sulfuric Acid of Various Concentrations

A 97% H_2SO_4 solution (1 ml) containing VII (1.123 mg) was heated at 80 °C for 2 h and the solution (50 μl) was added to H_2SO_4 (4 ml) of various concentrations containing H_2SeO_4. The final concentration of H_2SeO_4 was 0.0030% and those of H_2SO_4 were: 1 (60%), 2 (57%), 3 (54%), 4 (51%), and 5 (46%).

sulfuric acid were diluted similarly with the same acid at various concentrations in order to observe the absorbances at 482 and 600 nm. As shown in Fig. 4, where the acid-strength is expressed in terms of Hammett's acidity function (H_0), the formation of χ-482 and χ-600 increased gradually with decrease in the strength. This may indicate that these chromo- and fluorophores are produced from χ-301 (X) through its conjugate base (XI). When a solution of X in 97% sulfuric acid was diluted with an aqueous or ethanol solution of selenic acid, the formation of χ-482 was retarded but that of χ-600 was accelerated, indicating competitive transformation of these species from χ-301. The dication (X) changed increasingly to χ-600 with increase in the concentration of selenic acid (Fig. 5) and with decrease in the acid-strength of the mixture of sulfuric and selenic acids (Fig. 6). The transformation of χ-301 to χ-600 may, therefore, involve the oxidative process through the conjugate base (XI) of X. The decrease in the acid-strength of the solution may be essential to displace the equilibrium toward XI, if these changes of χ-301 to χ-482 and χ-600, which were caused by diluting the reaction mixture with water or ethanol as described above, are to occur.

A solution of VII in 97% sulfuric acid was heated at 80 °C for 2 h to give a solution of χ-301 (X), which was then diluted with two volumes of ethanol. When the alcoholic solution of χ-482 thus obtained was poured into an excess of ice-water, an epimeric mixture of 17ξ-isopropyl-18-nor-13ξ-androsta-4,6,8(14)-trien-3-one (XII) was obtained as a yellow oil, *m/e*: 296 (M^+), $\lambda_{max}^{EtOH}=349$ nm ($\varepsilon=25500$), $\nu_{C=O}$: 1655 cm^{-1}, 10% yield. The ^{1}H-NMR spectrum of the oil gave rather broad signals due to the vinyl protons of C(4), C(6), and C(7) at 5.73, 6.02, and 6.61 ppm, respectively, and two methyl signals due to the isopropyl group at 0.89 and 0.99 ppm (Table I). The ^{13}C-NMR spectrum gave signals due to seven sp^2 carbon atoms, C(3), C(4), C(5), C(6), C(7), C(8), and C(14), and also signals due to C(4), C(6), and C(7) of the isomer. There can be four isomers of XII due to the epimers at C(13) and C(17). Tóth *et al.*[8] isolated one of them, 17β-isopropyl-18-nor-13β-androsta-4,6,8(14)-trien-3-one, from the reaction mixture of VII and the sulfuric acid–ethanol system. A solution of X in 97% sulfuric acid was diluted with two volumes of ethanol containing 4% selenic acid to give a solution of χ-600, which, on treatment with ice-water, gave XIII as a brown oil, *m/e*: 294 (M^+), $\lambda_{max}^{EtOH}=$ 412 nm ($\varepsilon=21000$), $\nu_{C=O}$: 1650 cm^{-1}, 9% yield. The ^{1}H-NMR spectrum of this oil gave signals due to the vinyl protons of C(4), C(6), and C(7) at 5.73, 6.01, and 6.63 ppm, respectively, and methyl signals (non-equivalent) due to the isopropyl group at 1.07 and 1.04 ppm (Table I). The ^{13}C-NMR spectrum gave signals due to nine sp^2 carbon atoms, C(3), C(4), C(5), C(6), C(7), C(8), C(13), C(14), and C(17). These results indicate that the structure of the brown oil is 17-isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one (XIII).

As expected, the trienone (XII) and tetraenone (XIII) thus obtained instantly gave absorption maxima at 482 ($\varepsilon=38900$)[13] and 600 ($\varepsilon=52100$)[13] nm and emission maxima at 527 and 612 nm, respectively, when these enones were dissolved in a mixture (1 : 2, v/v) of sulfuric acid and ethanol (Fig. 7). The trienone (XII) was dissolved in 60% deuteriosulfuric acid and after 5 min, the ^{1}H-NMR spectra of the mixture gave signals due to the vinyl protons of C(4), C(6), and C(7), which were affected by the positive charge and shifted to the lower field (0.81, 0.61, and 0.98 ppm) than those shown in deuteriochloroform (Fig. 8). These values agree well with those shown by IV (Table I). These results may indicate that XII and XIII are the conjugate bases of the hydroxyalkatrienyl cation χ-482 (XIV) and the hydroxyalkatetraenyl cation χ-600 (XV), respectively.

Since the unsaturated steroidal ketones (XII and XIII) can be protonated to give their conjugate acids, the pK_a values of the acids thus formed may be regarded as an indicator of the stability of the cations (XIV and XV), though these ketones are not the Hammett's bases. The reported method[6] gave the pK_a values of XIV and XV as -4.6 and -3.8, which are in good agreement with those of the corresponding cations IV (-4.3)[5] and V (-3.2). The cation χ-482 (XIV) became unstable with increase in the acid-strength of the system, so that it was

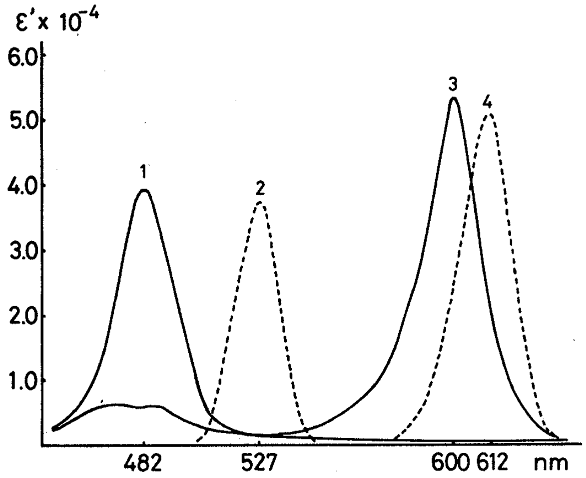

Fig. 7. Absorption and Fluorescence Spectra of χ-482 (XIV) and χ-600 (XV) in Sulfuric Acid–Ethanol Mixture (1 : 2, v/v)

XII or XIII (40 μg) was dissolved in 4 ml of H_2SO_4–ethanol (1 : 2, v/v) mixture. 1: Absorption spectrum of χ-482 (XIV). 2: Fluorescence spectrum of χ-482 with excitation at 467 nm. 3: Absorption spectrum of χ-600 (XV). 4: Fluorescence spectrum of χ-600 with excitation at 592 nm.

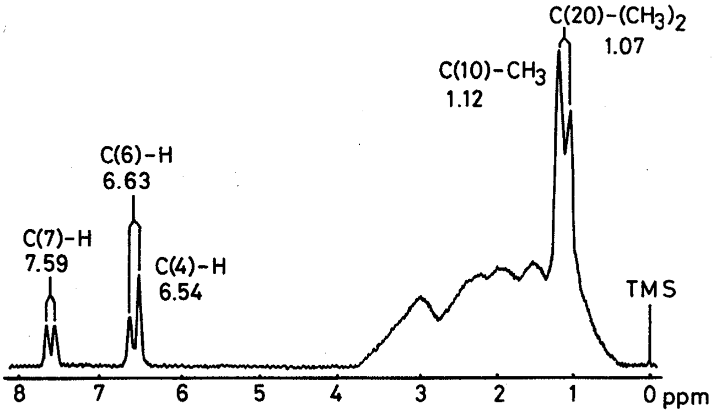

Fig. 8. ^{1}H-NMR Spectrum of χ-482 (XIV) in 60% Deuterio Sulfuric Acid

XII (40 mg) was dissolved in 0.5 ml of 60% D_2SO_4 (5 min).

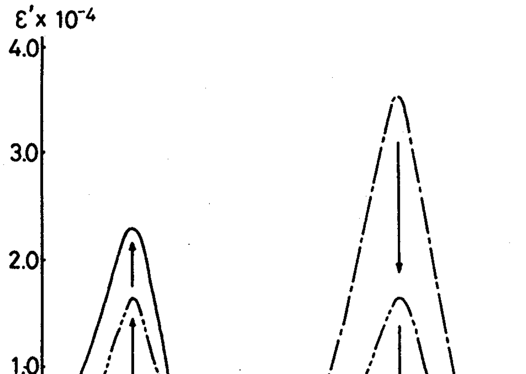

Fig. 9. Absorption Spectra of XII in 70% Sulfuric Acid

XII (40 μg) was dissolved in 70% H_2SO_4 (4 ml). – – –, 3 min; – - – -, 30 min; ——, 120 min.

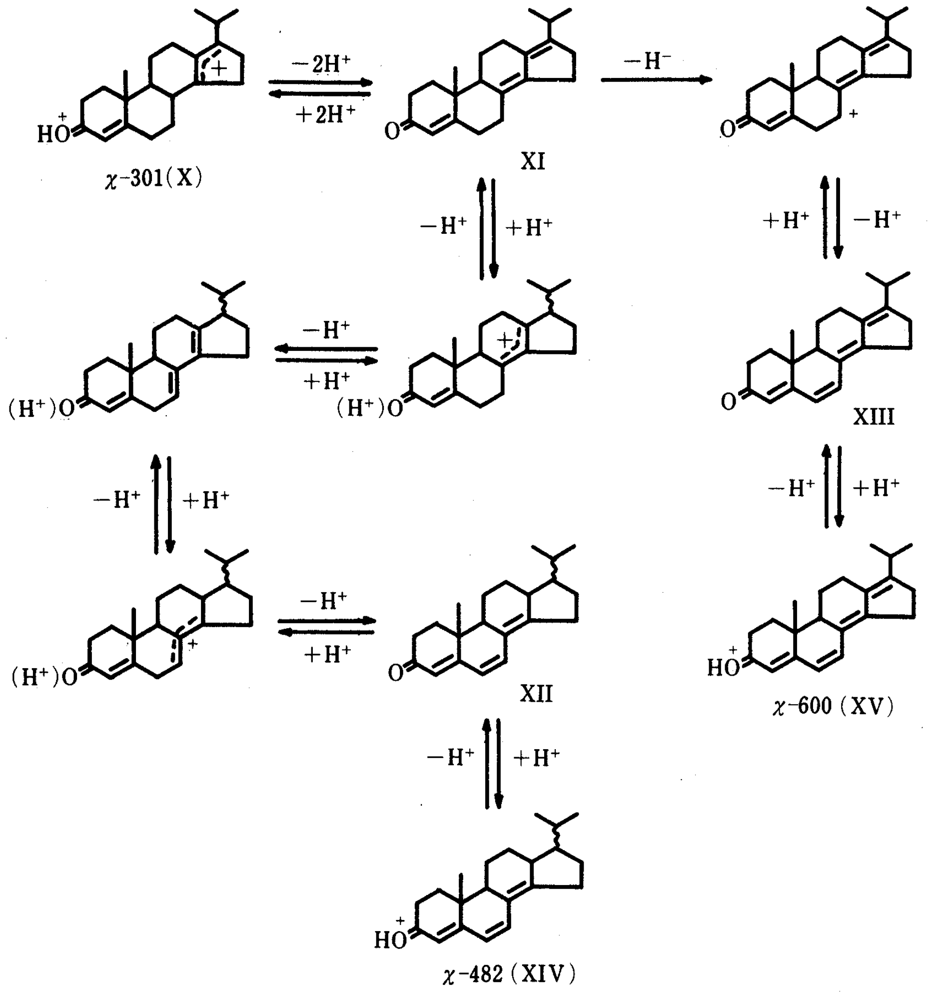

Chart 3. Mechanism of the Formation of Chromo- and Fluorophoric χ-482 and χ-600

transformed gradually in 70% sulfuric acid to the species showing an absorption maximum at 301 nm (Fig. 9). This change occurred instantly in 97% sulfuric acid, giving the same ^{1}H-NMR spectrum as that of χ-301. Thus, χ-482 is in an acid-base equilibrium with both its conjugate base (XII) and the dication χ-301, depending upon the acid-strength of the equilibrium system. The same relationship was also observed between III and IV.[6)]

It may be concluded from these results, that the transformation of the dication χ-301 to the chromo- and fluorophores χ-482 and χ-600 proceeds as shown in Chart 3. The stable χ-301 (X) formed from VIII in hot concentrated sulfuric acid becomes unstable with decrease in the acid-strength of the solution and is transformed to the cation χ-482 (XIV) through its conjugate base (XI). The transformation is reversible and the equilibrium lies to the side of X, when the sulfuric acid concentration is above 70%. Oxidation of XI, on the other hand, can occur competitively to give the cation χ-600 (XV). The color and fluorescence reaction of progesterone (VII) with sulfuric acid was, thus, shown to proceed by a mechanism similar to that in the reaction of testosterone (I).[1b)] These results may be important for elucidation of the mechanisms of the color and fluorescence reactions of other unsaturated ketosteroids with sulfuric acid. A similar study of corticosteroid is in progress.

Experimental

General Methods——Infrared (IR) spectra were measured with a JASCO A-102 recording spectrometer. Mass spectral (MS) measurements were run on a JEOL JMS-P-300 spectrometer. Absorption and fluorescence spectra were measured with a Shimadzu UV-220 recording spectrometer and a Hitachi MPF-3 fluorescence spectrometer, respectively, at room temperature unless otherwise noted. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX100 FT spectrometer at 100 MHz or a Hitachi R-20-B spectrometer at 60 MHz with tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were measured with a JEOL JNM-FX100 FT spectrometer at 25.0 MHz using tetramethylsilane as an internal standard. For preparative thin-layer chromatography (TLC), silica gel (Wakogel B-5F) was used as an adsorbent.

Materials——Progesterone (VII) was purchased from Sigma Chemical Co. and used after recrystallization. Sulfuric acid (super special grade, 97.2% w/w, Wako Pure Chemical Industries Ltd.) and sodium 3-(trimethylsilyl)propanesulfonate (Merck) were obtained commercially and used without further purification.

NMR Spectra in Sulfuric Acid——Sulfuric acid (60 or 97%, 0.5 ml) was added to a dried sample (40 mg) and the mixture was shaken vigorously to make a homogeneous solution. ^{1}H-NMR spectra were recorded at 35 °C. ^{13}C-NMR spectra were obtained by the Fourier transform method at 35 °C using tetramethylsilane in an external capillary as a reference. The deuterium signal of D_2O was used as a heteronuclear lock in the measurement of ^{13}C-NMR spectra. The solvent effects of sulfuric acid on chemical shifts were shown to be within 0.15 ppm for ^{1}H and 0.7 ppm for ^{13}C, based on the measured differences between external tetramethylsilane and sodium 3-(trimethylsilyl)propanesulfonate in 0—97% sulfuric acid.

Measurement of pK_a Values of Hydroxyalkatrienyl Cation (XIV) and Hydroxyalkatetraenyl Cation (XV)——An isooctane solution of XII or XIII (20 μg in 5 ml) was shaken for 30 s with an equal volume of sulfuric acid of various concentrations, and the absorption intensity at 327 nm for XII or 376 nm for XIII of the organic layer was measured and plotted against the H_0 value of the sulfuric acid. The apparent pK_a value of XIV or XV was determined, assuming that it corresponds to H_0 of the sulfuric acid and that a half of XII or XIII is present in the organic solvent.

Isolation of the Conjugate Base (XII) of χ-482 (XIV)——A mixture of sulfuric acid (97%, 3 ml) and VII (328 mg) was shaken vigorously to make a homogeneous solution, which was heated at 80 °C for 2 h. The reaction mixture was diluted with ethanol (6 ml) under ice-cooling to give a red-brown solution, which was gradually poured into an excess of ice-water under vigorous stirring. The whole was extracted with chloroform (200 ml × 3). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue thus obtained was subjected to preparative TLC developed with benzene–acetone (8 : 1, × 2). Elution of the adsorbent corresponding to the spot of *Rf* 0.56 with chloroform gave 17ξ-isopropyl-18-nor-13ξ-androsta-4,6,8(14)-trien-3-one (XII) as a yellow oil (30 mg), which was a mixture of at least two epimeric isomers as judged from its ^{13}C-NMR spectrum. The stereochemical configurations at C(13) and C(17) of these epimers remain uncertain. $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 349 (25500). MS *m/e*: 296 (M$^+$). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 1655 (C=O). ^{1}H-NMR and ^{13}C-NMR data are shown in Tables I and II, respectively. In the ^{1}H-NMR spectrum, vinyl proton signals at C(4), C(6), and C(7) appeared as slightly broad signals.

Isolation of the Conjugate Base (XIII) of χ-600 (XV)——A mixture of sulfuric acid (97%, 3 ml) and VII (526 mg) was shaken vigorously to make a homogeneous solution, which was heated at 80 °C for 2 h. The reaction mixture was diluted with ethanol (6 ml) containing selenic acid (4%) under ice-cooling to give a deep purple solution. The solution thus obtained was worked-up and subjected to preparative TLC in the same manner as described above. Elution of the adsorbent corresponding to the spot of *Rf* 0.51 with chloroform gave 17-isopropyl-18-norandrosta-4,6,8(14),13(17)-tetraen-3-one (XIII) as a red-brown oil. $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 412 (21000). MS *m/e*: 294 (M$^+$). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 1650 (C=O). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II.

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References and Notes

1) *a)* This paper constitutes Part XVI of the series entitled "Chromogenic Reactions of Steroids with Strong Acids"; *b)* Part XV: H. Takagi, T. Miura, and M. Kimura, *Chem. Pharm. Bull.*, **30**, 3493 (1982).
2) M. Kimura, M. Kawata, K. Akiyama, K. Harita, and T. Miura, *Chem. Pharm. Bull.*, **21**, 1720, 1741 (1973); M. Kimura, K. Akiyama, and T. Miura, *ibid.*, **22**, 643 (1974); T. Miura and M. Kimura, *ibid.*, **24**, 181 (1976); *idem, ibid.*, **25**, 1042 (1977); *idem, ibid.*, **26**, 171 (1978).
3) M. Kimura and K. Harita, *Chem. Pharm. Bull.*, **21**, 1205, 1235, 1261 (1973).
4) T. Miura, H. Takagi, K. Harita, and M. Kimura, *Chem. Pharm. Bull.*, **27**, 452 (1979).
5) T. Miura, H. Takagi, and M. Kimura, *Chem. Pharm. Bull.*, **27**, 783 (1979).
6) H. Takagi, M. Kokuryo, T. Miura, and M. Kimura, *Bunseki Kagaku*, **30**, 191 (1981).
7) T. Miura, H. Takagi, K. Adachi, and M. Kimura, *Chem. Pharm. Bull.*, **30**, 3154 (1982).
8) G. Tóth, A. Szabo, D. Mullur, and G. Snatzke, *Collect. Czech. Chem. Commun.*, **43**, 165 (1978).
9) Y. Asahi, M. Ohnishi, and N. Nakajima, *Bunseki Kagaku*, **28**, 540 (1979).
10) A. M. Smoczkiewicz and R. M. Zalewski, *Steroids*, **12**, 391 (1968).

11) *a)* N. C. Deno, H. G. Richey, J. N. Friedman, J. D. Hodge, J. J. Houser, and C. V. Pittman, *J. Am. Chem. Soc.*, **85**, 2991 (1963); ^{1}H-NMR data (ppm, in 96% H_2SO_4) for VI: 1.43 (d, CH_3 of isopropyl group), 7.68 (C(2)–H), 2.99 (C(3)–CH_3), 3.54 (C(4)– or C(5)–H); *b)* N. C. Deno, J. Bollinger, K. Hafer, J. D. Hodge, and J. J. Houser, *J. Am. Chem. Soc.*, **85**, 2998 (1965).

12) A. R. Butler and H. A. Jones, *J. Chem. Soc., Perkin Trans. 2*, **1976**, 963.

13) The yields of χ-482 and χ-600 from progesterone (VII) were calculated by using these ε values and were as follows: χ-482 (66%) and χ-600 (18%) under the conditions given in Fig. 1 and χ-482 (10%) and χ-600 (7%) under those in Fig. 4.