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Chromogenic Reactions of Steroids with Strong Acids. IX.[1] Behavior of Estrone Methyl Ether in Concentrated Sulfuric Acid

Toshiaki Miura and Michiya Kimura

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

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The behavior of estrone methyl ether (V) in strong acid was studied, in order to elucidate the mechanism of the Kober color reaction. When V was dissolved into concentrated sulfuric acid, a maximum absorption gradually appeared at 465 nm which was then transferred to 452 nm with elapse of time. On the other hand, 3-methoxy-17α-methylestra-1,3,5(10),9(11)-tetraen-17β-ol (XIIIb) immediately gave the chromophoric cation χ-465 (VIIb) in concentrated sulfuric acid. Similarly, 3-methoxyestra-1,3,5(10),-9(11)-tetraen-17β-ol (XIIIa) gave the cation χ-364 (XIVa) which changed in turn to χ-465 (VIIa). Sulfonation gradually occurred at C(2)s of VIIa and VIIb in the same acid to give the corresponding cations XIXa and XIXb; the maximum absorption at 465 nm altered to 452 nm. The mechanism of the conversion of V into the C(2)-sulfonated χ-465 (XIXa) was elucidated from these behavior of XIIIa and XIIIb in concentrated sulfuric acid.

Keywords——color reaction; estrone methyl ether; sulfuric acid; Kober reaction; steroidal carbocation; rearrangement; sulfonation; NMR

The utility of urinary estriol determinations during the later stages of pregnancy is well established as an index of fetal viability.[3] In most of the methods reported, the Kober color reaction[4] and its modifications[5] have long been utilized for the assay of steroidal estrogens from body fluids in clinical chemistry. In spite of the widespread interest in this assay, its chemistry has been little studied[6–12] and has remained obscure. In order to clarify the mechanism of the Kober reaction, we have investigated the behavior of estrane derivatives in strong acids. It was demonstrated that some 3-methyl ethers of estrogens having a hydroxyl function at C(17) position, such as β-estradiol methyl ether (3-methoxyestra-1,3,5(10)-trien-17β-ol, I), α-estradiol methyl ether (3-methoxyestra-1,3,5(10)-trien-17α-ol, II) and 3-methoxy-17α-methylestra-1,3,5(10)-trien-17β-ol (III), were converted into the carbocations[13] (IVa,b) in concentrated sulfuric acid (Chart 1).[1,8,9] On the contrary, estrone methyl ether (3-methoxyestra-1,3,5(10)-trien-17-one, V) in the same conditions showed a series of spectral change

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different from that of these 17-hydroxyestranes and gave a sulfonated product instead, as previously reported.[9] We present in this paper an explanation of such a characteristic behavior shown by the methyl ether of estrone which is one of the main steroidal estrogens and has long been assayed also by using the Kober reactions.

Results and Discussion

We reported previously that, when II was dissolved in concentrated sulfuric acid (97.2%), a stable carbocation (IVa) showing the absorption maximum at 372 nm was immediately formed by removal of the hydroxyl group at C(17), followed by the Wagner-Meerwein rearrangement of the methyl group from C(13) to C(17) and subsequent hydride shifts.[8] Although IVa was stable in concentrated sulfuric acid for long time at room temperature, it was gradually transformed into a chemical species showing an absorption maximum at 465 nm on dilution of the acid solution with water. The chromophoric χ-465 (designation for the species indicating a maximum absorption at 465 nm) was found to be the carbocation (VIIa) which was produced from the conjugate base (VIa) of IVa by the oxidation with sulfuric acid (Chart 1).[1] On the other hand, the concentrated sulfuric acid solution of estrone methyl ether (V) showed

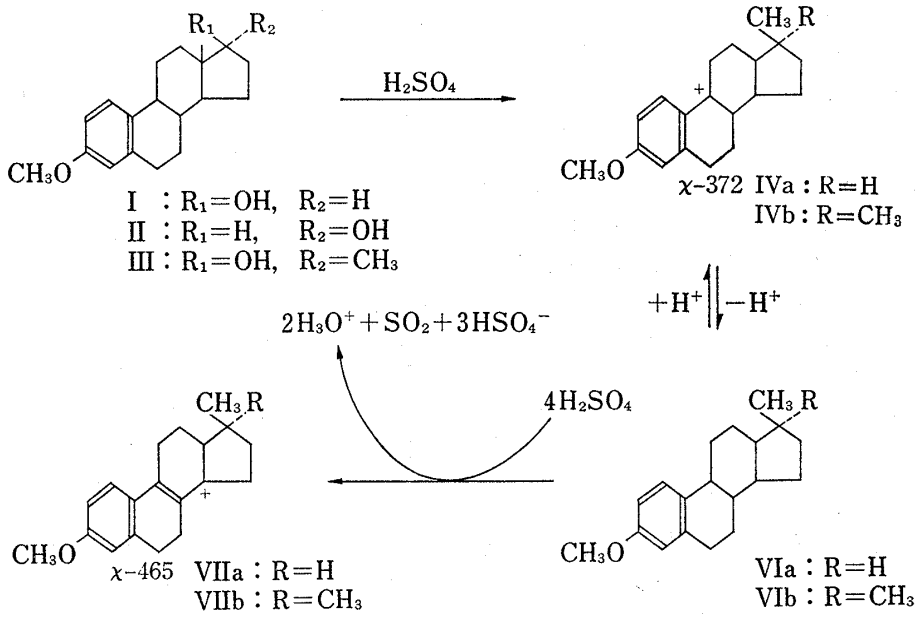


Chart 1

gradually an absorption at 465 nm without any detectable absorption at 372 nm and its apparent molar extinction coefficient (ε') was 15000 even after three hours (Fig. 1).[9] Since the molar extinction coefficient of χ-465 (VIIa, b) is 64000,[1] formation of this chromophore from V seems to be slow. Furthermore, it was found that the absorption maximum of the chromophoric χ-465 derived from V shifted from 465 to 452 nm with the passage of time (Fig. 1). Our investigation was focussed, firstly, on the mechanism of formation of the carbocations from V and, secondly, on the cause of the shift of its absorption maximum.

Formation of Carbocations from Estrone Methyl Ether (V)

As in the cases of 17-hydroxyestranes (I, II), V was transformed into 17-methyl-18-norestrane derivatives through the rearrangement of the methyl group from C(13) to C(17) under conditions of the first stage of the Kober reaction (78% H_2SO_4, 100°, 40 min).[7] This fact seemed to indicate that the rearrangement is essential for the formation of the chromo-

phoric χ-465 from V. Olah, *et al.*[14] observed the nuclear magnetic resonance (NMR) spectra of protonated aliphatic ketones in FSO_3H–SbF_5–SO_2 at −60° and found that the positive charge resides mainly on oxygen (VIIIa) and the resonance form VIIIb (dialkylhydroxy carbenium ion) is of lesser significance (Chart 2). Thus, of the two resonance forms IXa and IXb (Chart 3) the latter may be of lesser significance. The nucleophilic rearrangement of the methyl group from C(13) to C(17) may, therefore, be slow and a rate determining-step for the formation of the chromophoric χ-465 (VIIa). Once the methyl group migrates, VIIa may immediately be formed by subsequent rapid hydride shifts, dehydration of the tertiary hydroxyl group, and deprotonation (X→XI→XIIa—VIIa). In fact, no maximum absorption at 372 nm, which is shown by the carbocation (IVa)

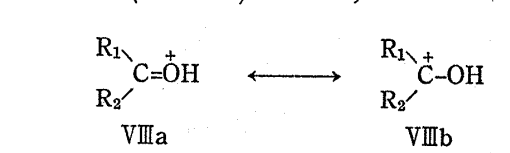


Chart 2

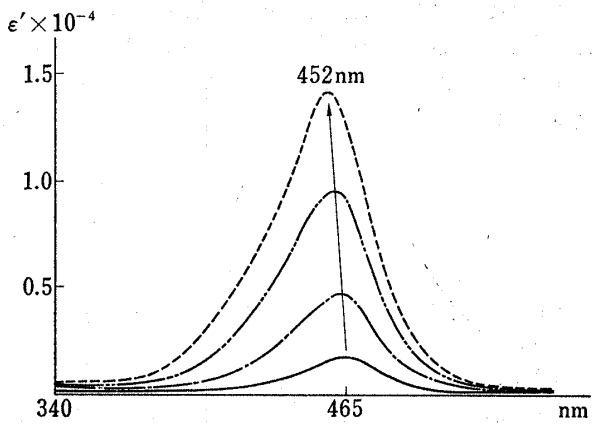


Fig. 1. Absorption Spectra of Estrone Methyl Ether(V) in Concentration Sulfuric Acid

147 μg of V in 10 ml of 97.2% H_2SO_4 at 25°.
———: 10 min, — — — —: 60 min,
— — —: 30 min, ————: 180 min.
ε′=apparent molar extinction coefficient.

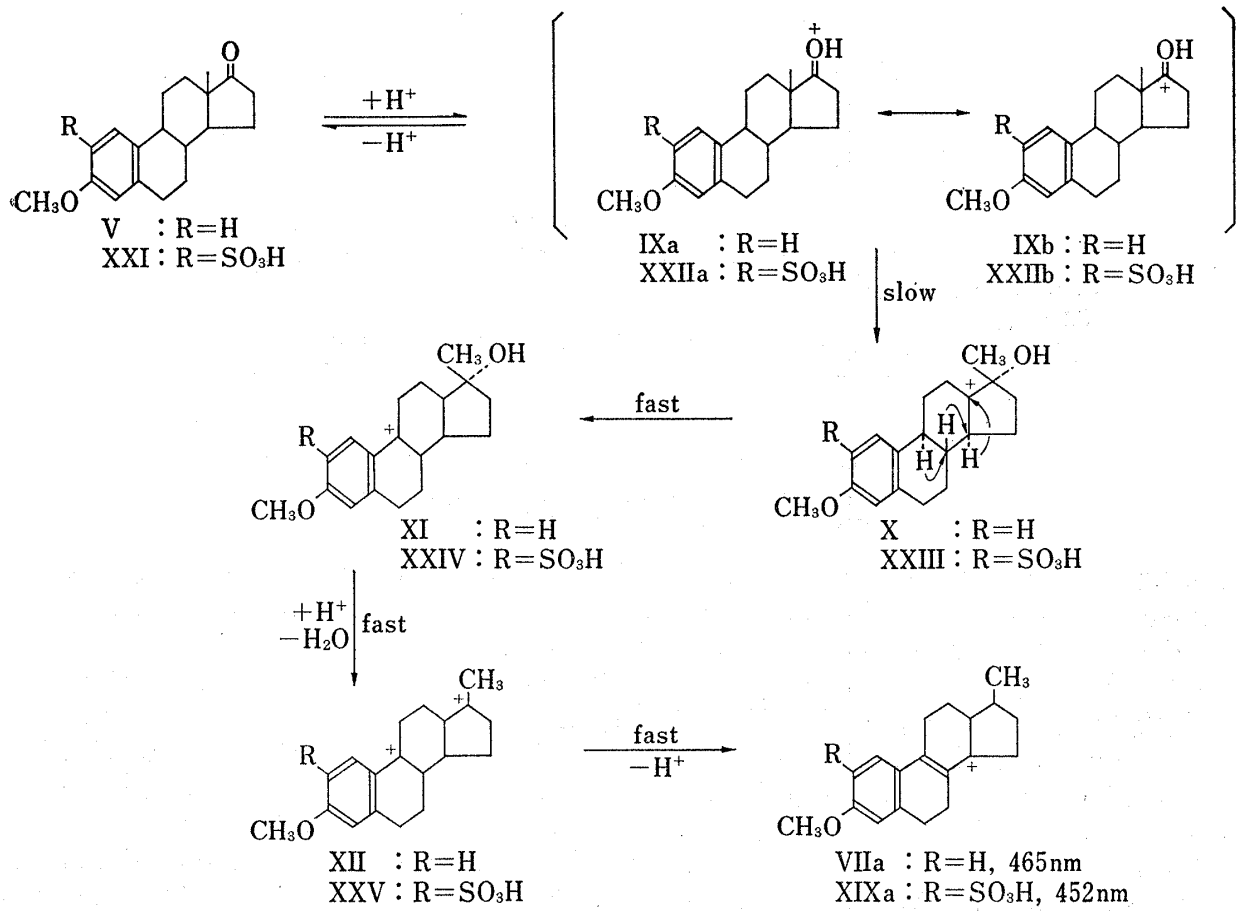


Chart 3

14) G.A. Olah, M. Calin, and D.H. O'Brien, *J. Am. Chem. Soc.*, **89**, 3586 (1967).

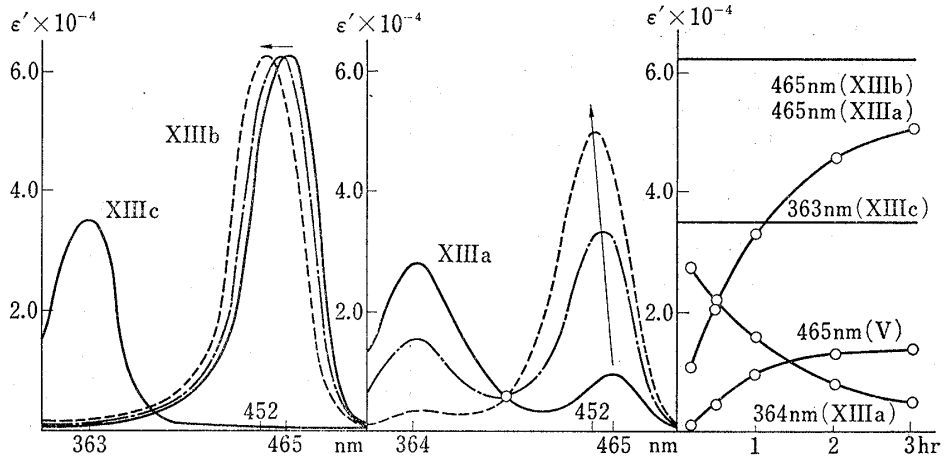
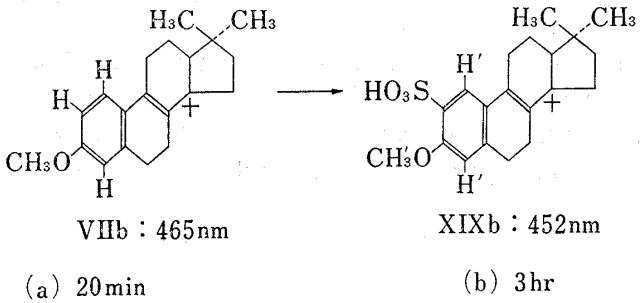


Fig. 2. Absorption Spectra of XIIIa, XIIIb and XIIIc in Concentrated Sulfuric Acid at 25°

Steroid: 25—50 μg, 97.2% H_2SO_4: 5 ml.
————: 10 min, — - —: 60 min, ---------: 180 min.
ε =apparent molar extinction coefficient.



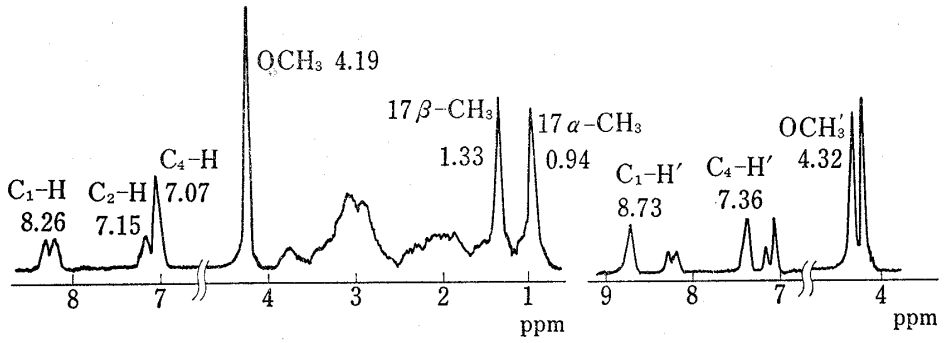


Fig. 3. NMR Spectra of XIIIb in Concentrated Sulfuric Acid

40 mg of XIIIb in 0.5 ml of 97.2% H_2SO_4, 60 Mc, 35°
ppm from external capillary tetramethylsilane.

and might be expected to hold for XI, was observed but an absorption at 465 nm appeared gradually, when V was dissolved into concentrated sulfuric acid. These results may reasonably be interpreted by the mechanism indicated in Chart 3.

In order to confirm the proposed reaction mechanism, 3-methoxy-17α-methylestra-1,3,5(10),9(11)-tetraen-17β-ol (XIIIb), which has a structure equivalent to that of the conjugate base of XI, was synthesized and its behavior in concentrated sulfuric acid was investigated. Dissolution of XIIIb into the acid showed no absorption at 372 nm but immediately an absorption at 465 nm (ε=64000) as expected (Fig. 2). NMR spectrum of the tetraene (XIIIb) in concentrated sulfuric acid (Fig. 3a) was identical with that of the carbocation (VIIb) previously reported.[1] When XIIIb was dissolved into concentrated sulfuric acid and allowed to stand at room temperature for four minutes, the conjugate base of VIIb was obtained in 80% yield as a mixture of XV and XVI in a ratio of 3:1 (Chart 4). The mixture was identified by comparison of its spectral data with those of an authentic sample[1] and dehydrogena-

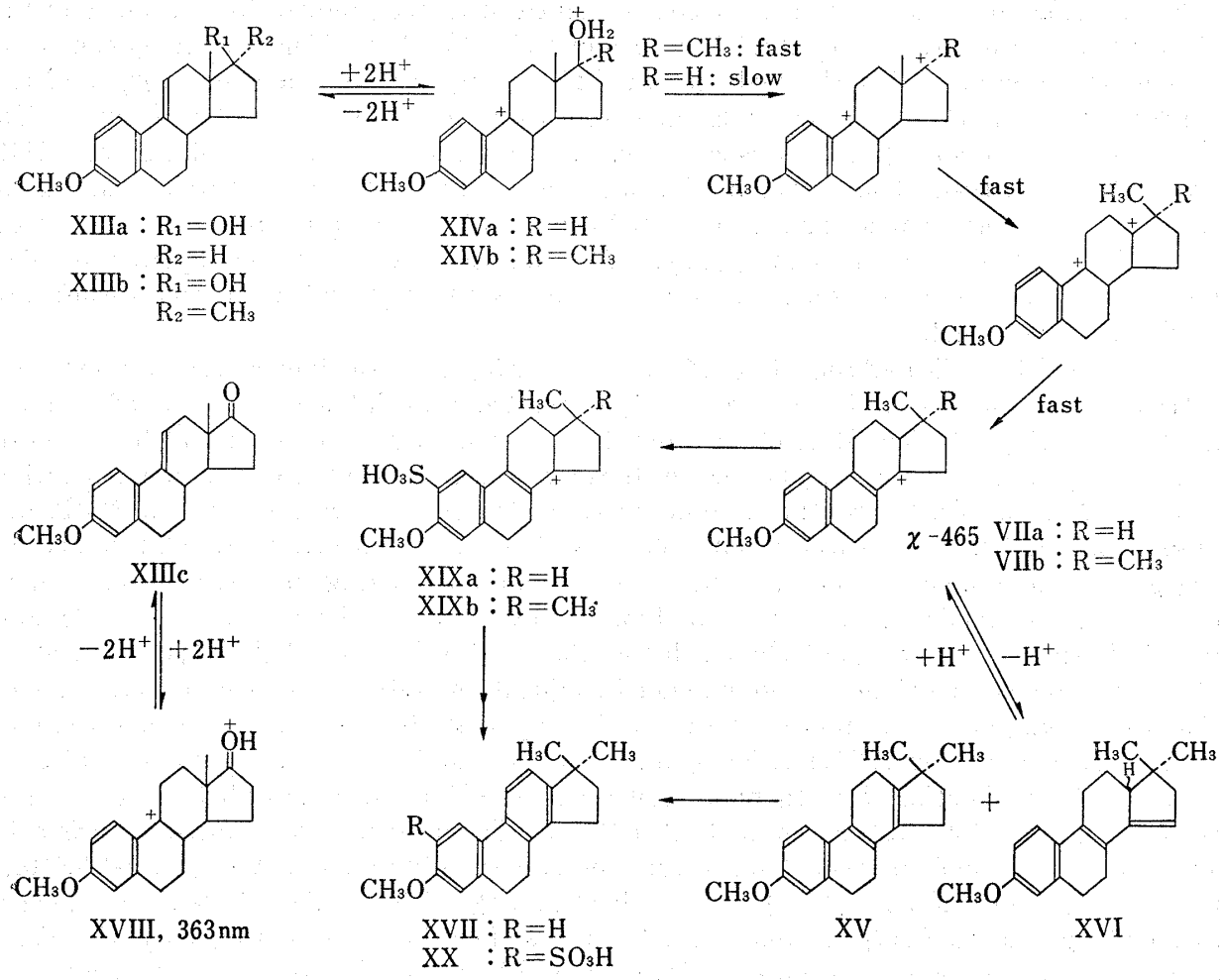


Chart 4

tion to 3′,3′-dimethyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene (XVII) over 5% palladium on charcoal in boiling ethanol. These results led to the conclusion that the chromophoric χ-465 produced from XIIIb was the carbocation VIIb. Dissolution of 3-methoxyestra-1,3,5(10),9(11)-tetraen-17β-ol (XIIIa) into concentrated sulfuric acid showed a maximum absorption at 364 nm which transferred gradually to 465 nm with elapse of time (Fig. 2). The gradual formation of χ-465 from XIIIa may be due to the low velocity in the dehydration of the secondary hydroxyl group at C(17)β position.[15] Since the carbocation (XVIII) derivable from 3-methoxyestra-1,3,5(10),9(11)-tetraen-17-one (XIIIc) gave a maximum absorption at 363 nm (ε=35000) as shown in Fig. 2,[1] the chemical species giving a maximum at 364 nm with XIIIa may be the carbocation (XIVa) which may gradually transfer to the chromophoric χ-465 (VIIa) by removal of the hydroxyl group at C(17)β, followed by the migration of the angular methyl group and deprotonation (Chart 4). Based on such behavior of XIIIa and XIIIb in concentrated sulfuric acid, the reaction mechanism indicated in Chart 3 may be plausible.

Sulfonation and Blue Shift of the Absorption Maximum at 465 nm

The shift of the absorption maximum of χ-465 from 465 to 452 nm was observed in the cases of XIIIa and XIIIb (Fig. 2) as was shown by V (Fig. 1). NMR spectrum of XIIIb in concentrated sulfuric acid obtainable at twenty minutes after mixing was identical with that given by VIIb as shown in Fig. 3a. For three hours after mixing, the singlet signals at 8.73,

15) D.N. Kirk and M.P. Hartshorn, "Steroids Reaction Mechanisms," Elsevier, Amsterdam, 1968, p. 261.

7.36, and 4.32 ppm continued to grow concomitantly with the gradual disappearance of the signals due to aromatic and methoxyl protons of VIIb (Fig. 3b). This spectral change may be best interpreted in that the hydrogen at C(2) was replaced by the sulfo group and the singlet signals at 8.73, 7.36, and 4.32 ppm were ascribable to C(1)–H, C(4)–H, and OCH_3 of C(2)-sulfonated χ-465 (XIXb), respectively. In fact, when the colored solution of XIIIb obtainable at forty-six hours after mixing was poured into water and allowed to stand for seven days at room temperature, a sulfonated compound was obtained from the solution by Amberlite XAD-2 resin chromatography. The compound was then methylated to give methyl 3′,3′-dimethyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene-6-sulfonate (XX) as colorless plates.

These results indicated that the chromophoric χ-465 (VIIb) was immediately formed from XIIIb (Fig. 2 and 3a), which was gradually converted into C(2)-sulfonated χ-452 (XIXb) as shown in Fig. 3b. The shift of an absorption maximum of χ-465 (VIIa, b) from 465 to 452 nm (Fig. 2) may, therefore, be due to its transformation into the C(2)-sulfonated cation χ-452 (XIXa, b). On the contrary, no appreciable changes were observed in the NMR and absorption spectra of the carbocations IVa, b and XVIII in concentrated sulfuric acid,[1,8] suggesting that the sulfonation did not occur at the aromatic rings of these cations. Such difference in the tendency to be sulfonated may be explained by the reason that the electron density in the aromatic ring of VIIa, b is higher than that of IVa, b and XVIII; delocalization of the positive charge along a more extended conjugation system was suggested by the fact that the signal due to C(2)-proton of VIa, b (7.15 ppm) shifted to higher field than those of IVa, b (7.19 ppm) and XVIII (7.25 ppm).[1] In the reaction of XIIIa with concentrated sulfuric acid, sulfonation may, therefore, not occur in XIVa and be initiated at the stage of χ-465 (VIIa).[16] When estrone methyl ether (V) was dissolved in concentrated sulfuric acid and allowed to stand at room temperature for twenty minutes, 3-methoxyestra-1,3,5(10)-trien-17-one-2-sulfonic acid (XXI) was obtained in 70% yield as methylester,[9] in which a carbonyl function at C(17) remained intact. The sulfonation of V at C(2) may, therefore, compete with the transformation of V into χ-465 (VIIa) and V may mostly be converted into the sulfonic acid XXI in an early period.

Taking these findings into consideration, it may be concluded that the color reaction of estrone methyl ether (V) with concentrated sulfuric acid characteristically proceeded as shown in Chart 3. Namely, the protonation at C(17)-carbonyl function and the sulfonation at C(2) may take place to form XXII which is then converted into XXIII by the slow methyl migration from C(13) to C(17). The subsequent rapid hydride shifts may lead XXIII to XXIV which is in turn converted into the sulfonated carbocation χ-452 (XIXa) through the dication XXV by rapid dehydration of the tertiary hydroxyl group and deprotonation, as in the case of XIIIb (Chart 4). A mere portion of V, on the other hand, may undergo a series of similar transformation without sulfonation to give the chromophoric χ-465 (VIIa) which is then sulfonated to yield χ-452 (XIXa).

Experimental[17]

3-Methoxyestra-1,3,5(10),9(11)-tetraen-17β-ol (XIIIa)——To a solution of 3-methoxyestra-1,3,5(10),9(11)-

16) In the reaction of 17-hydroxyestranes (I, II, III) resulting χ-465 (VIIa, b), the possibility of sulfonation may reasonably be ruled out, since the carbocation (IVa, b) formed initially was then treated in a diluted sulfuric acid at room temperature.[8]

17) All melting points were taken on a micro hot-stage apparatus and are uncorrected. For the preparative thin-layer chromatography (TLC) silica gel (Wakogel B-5-F) was used as an adsorbent. Ultraviolet (UV) and visible (V) spectra were recorded on Hitachi Model 3T spectrometer. NMR spectrum measurements were run on Hitachi Model R-20-B spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. Mass spectra (MS) were measured by Hitachi Model RMU-6R spectrometer.

tetraen-17-one (XIIIc, 43 mg) in MeOH (15 ml) was added $NaBH_4$ (30 mg) under ice-cooling and allowed to stand at room temperature for 1.5 hr. The reaction mixture was concentrated, diluted with water and extracted with ether. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness yielding pale yellow crystals, recrystallization of which gave XIIIa as colorless needles (30 mg) from MeOH, mp 68—70° (lit.[18] 71—73°). MS *m/e*: 284 (M^+). UV λ_{max}^{MeOH} nm (ε): 263 (19700). *Anal.* Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.11; H, 8.42. NMR ($CDCl_3$) δ: 0.80 (3H, s, 13-CH_3), 3.65 (1H, m, 17α-H), 3.73 (3H, s, OCH_3), 6.10 (1H, broad s, 11-H), 6.57 (1H, d, J=2.5 Hz, 4-H), 6.66 (1H, q, J_1=9 Hz, J_2=2.5 Hz, 2-H), 7.48 (1H, d, J=9 Hz, 1-H).

3-Methoxy-17α-methylestra-1,3,5(10),9(11)-tetraen-17β-ol (XIIIb)——To a solution of CH_3MgI in ether (40 ml) prepared from Mg turnings (800 mg) and CH_3I (8 ml) was added under nitrogen atmosphere at 0° dropwise a solution of XIIIc (347 mg) in ether–benzene (6: 1, 35 ml). After being stirred for 30 min at room temperature, the mixture was refluxed for 7 hr. To the cooled solution were added successively excess NH_4Cl, ice-water and 5% HCl. The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to give yellow oil (386 mg). The oily residue was submitted to the preparative TLC using benzene–EtOH (95: 5) as developing solvent. The adsorbent corresponding to the spot (Rf=0.31) was eluted with $CHCl_3$ and the eluate (colorless oil, 220 mg) was crystallized from MeOH to give XIIIb (170 mg) as colorless needles, mp 78.5—80.5°. MS *m/e*: 298 (M^+). $[\alpha]_D^{25°}$ +115° (c=0.09, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 263 (19400). *Anal.* Calcd. for $C_{20}H_{26}O_2$: C, 80.49; H, 8.78. Found: C, 80.53; H, 8.84. NMR ($CDCl_3$) δ: 0.90 (3H, s, 13-CH_3), 1.25 (3H, s, 17α-CH_3), 3.75 (3H, s, OCH_3), 6.13 (1H, broad s, 11-H), 6.58 (1H, d, J=2.5 Hz, 4-H), 6.68 (1H, q, J_1=9 and J_2=2.5 Hz), 7.50 (1H, d, J=9 Hz, 1-H).

Reaction of XIIIb with Concentrated Sulfuric Acid——To a dried sample of XIIIb (30 mg) was added 97.2% H_2SO_4 (5 ml) with vigorous stirring. The reaction mixture became homogeneous and orange with green fluorescence. The stirring was continued for 5 min at room temperature, and the colored solution was dropped into vigorously stirred ice-water (100 ml) and then extracted with benzene. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness yielding a crystalline residue (26 mg). Recrystallization of the residue from EtOH gave a 3: 1 mixture of XV and XVI[1] as colorless prisms, mp 98—100°. MS *m/e*: 280 (M^+), 265 (M^+-CH_3). UV λ_{max}^{EtOH} nm (ε): 313 (17600). *Anal.* Calcd. for $C_{20}H_{24}O_2$: C, 85.66; H, 8.63. Found: C, 85.48; H, 8.69. NMR ($CDCl_3$) δ: 0.88 (6H, s, 17-*gem* CH_3 of 13α- or 13β-isomer of XVI), 1.06 (6H, s, 17-*gem* CH_3 of XV), 1.16 (6H, s, 17-*gem* CH_3 of 13α- or 13β-isomer of XVI), 3.75 (3H, s, OCH_3), 5.52 (1H, broad s, 15-H of XVI), 6.60—7.30 (3H, arom.).

3′,3′-Dimethyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene (XVII)——A mixture of XV+XVI (3: 1, 20 mg) and 5% palladium-on-charcoal (70 mg) in EtOH (30 ml) was refluxed for 4 hr. The reaction mixture was cooled and then filtrated. The filtrate was concentrated under reduced pressure, and the residue was crystallized from EtOH to give XVII (13 mg) as colorless needles, mp 99—100°. MS *m/e*: 278 (M^+), 263 (M^+-CH_3). UV λ_{max}^{EtOH} nm (ε): 282 (26900). *Anal.* Calcd. for $C_{20}H_{22}O$: C, 86.28; H, 7.97. Found: C, 86.39; H, 8.02. NMR ($CDCl_3$) δ: 1.37 (6H, s, 3′-*gem* CH_3), 1.94 (2H, t, J=8 Hz, 2′-H), 2.79 (4H, s, 9,10-H), 2.85 (2H, t, J=8 Hz, 1′-H), 3.80 (3H, s, OCH_3), 6.73 (1H, d, J=2.5 Hz, 8-H), 6.81 (1H, q, J_1=9 and J_2=2.5 Hz, 6-H), 7.06 (1H, d, J=8 Hz, 3-H), 7.56 (1H, d, J=8 Hz, 4-H), 7.66 (1H, d, J=9 Hz, 5-H).

Sulfonation at C-2 of the Chromophore (VIIb) in Concentrated Sulfuric Acid——To a dried sample of XIIIb (40 mg) was added 97.2% H_2SO_4 (0.5 ml) with vigorous shaking to give a homogeneous solution. NMR spectra were recorded with external capillary tetramethyl silane (TMS) as a reference at 35° (Fig. 3). After 46 hr, the mixture was dropped into vigorously stirred ice-water (30 ml) and allowed to stand for 7 days at room temperature. The acidic solution was percolated through a column packed with Amberlite XAD-2 resin (1000 ml) and then washed with water (1000 ml). Evaporation of the solvent from the first fraction eluted with MeOH (1000 ml) left a residue which was then methylated with CH_2N_2, giving yellow oil (25 mg). The oil was submitted to the preparative TLC using benzene–EtOH (95: 5) as developing solvent. The adsorbent corresponding to the spot (Rf=0.45) was eluted with AcOEt and the eluate (crystalline residue, 18 mg) was recrystallized from EtOH to give methyl 3′,3′-dimethyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene-6-sulfonate (XX) as colorless plates, mp 154—157°. MS *m/e*: 372 (M^+), 357 (M^+-CH_3). UV λ_{max}^{EtOH} nm (ε): 282.5 (25900). *Anal.* Calcd. for $C_{21}H_{24}O_4S$: C, 67.73; H, 6.50; S, 8.59. Found: C, 67.49; H, 6.54; S, 8.68. NMR ($CDCl_3$) δ: 1.39 (6H, s, 3′-*gem* CH_3), 1.98 (2H, t, J=7 Hz, 2′-H), 2.90 (2H, t, J=7 Hz, 1-H), 2.88 (4H, s, 9,10-H), 3.84 (3H, s, SO_3CH_3), 4.02 (3H, s, OCH_3), 6.97 (1H, s, 8-H), 7.15 (1H, d, J=9 Hz, 3-H), 7.67 (1H, d, J=9 Hz, 4-H), 8.31 (1H, s, 5-H).

Absorption Spectrum——To a dried sample was added 5 ml of 97.2% H_2SO_4 at room temperature and the mixture was shaken vigorously. Spectra of the homogeneous solution obtained were measured at 25°.

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