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Chromogenic Reactions of Steroids with Strong Acids. VII.[1] Reactions of A-Aromatic Steroids with Concentrated Sulfuric Acid[2]

Michiya Kimura and Toshiaki Miura

Faculty of Pharmaceutical Sciences, Hokkaido University[3]

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As in the case of 3-methoxy-17α-methylestra-1,3,5(10)-trien-17β-ol (IV),[1] the maximum absorption at 372 nm was immediately shown when 3-methoxyestra-1,3,5(10)-trien-17α-ol (V), 3-methoxyestra-1,3,5(10),16-tetraene (VI), or 3-methoxy-17β-methyl-18-norestra-1,3,5(10),13-tetraene (VII) was dissolved in 97.2% H_2SO_4. Colorless leaflets, mp 122—124°, were obtained in 90% yield when IV came into contact with the same acid at room temperature for 20 min. Ultraviolet, mass and nuclear magnetic resonance spectral data indicated this product to be 3-methoxy-17,17-dimethyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VIIIb). Similarly, the same reaction of V, VI, or VII gave 3-methoxy-17β-methyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VIIIa) in 80—90% yields, which also immediately shows maximum absorption at 372 nm in conc. H_2SO_4 (Chart 2). Formation of the stable carbocation III seemed to be slow in the case of estradiol-3-methyl ether (II). No χ-372 but χ-465[1] was formed in conc. H_2SO_4 with estrone methyl ether (I) which is in higher oxidation state than those of II, IV, V, VI, and VII (Fig. 1).

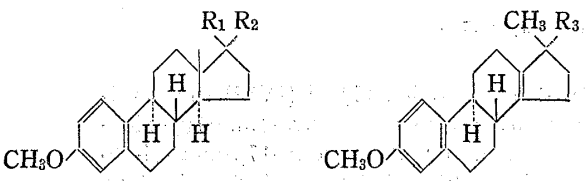
Since Kober[4] reported the specific color reaction for the phenolic steroids,[5] numbers of its modification have been studied and utilized for the analysis of various steroidal estrogens.[6] Mechanism of the Kober reaction, however, is not fully understood, though there appeared several reports lacking in the detailed chemistry.[7] The present authors have studied the specificity of Kober reaction,[8] structure elucidation of the products from the methyl ether (I) of estrone (3-hydroxyestra-1,3,5(10)-trien-17-one) and that (II) of estradiol

1) Part VI: M. Kimura, K. Akiyama, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **22**, 643 (1974).
2) Preliminary accounts of a part of this work have been published: M. Kimura, K. Akiyama, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **20**, 2511 (1972).
3) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
4) S. Kober, *Biochem. Z.*, **239**, 209 (1931); *idem*, *Biochem. J.*, **32**, 357 (1938).
5) L. Fieser and M. Fieser, "Steroids," Reinhold Publishing Co., New York, N. Y., 1959, p. 469.
6) W.S. Bauld, *Biochem. J.*, **56**, 426 (1954); J.B. Brown, *J. Endocrinol.*, **8**, 196 (1952); *idem*, *Biochem. J.*, **60**, 185 (1955); G. Ittrich, *Z. Physiol. Chem.*, **312**, 1 (1958); *idem*, *Acta Endocrinol.*, **35**, 34 (1960); R.I. Dorfmann, "Methods in Hormone Research," Vol. 1, Academic Press, New York, N.Y., 1962, p. 1 and see also reference therein.
7) W. Zimmermann, "Chemische Bestimmungsmethoden von Steroidhormonen in Körperfulüssigkeiten," Springer-Verlag, Berlin, 1955, p. 52; H.A. Jones and R. Hähnel, *Nature*, **215**, 1381 (1967); *idem*, *Steroids*, **13**, 693 (1969).
8) M. Kimura, M. Kawata, K. Akiyama, K. Harita, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **21**, 1720 (1973).

(estra-1,3,5(10)-triene-3,17β-diol), in this reaction,[9] and the behavior of various phenolic steroids in concentrated sulfuric acid.[1] It was thus postulated that the elimination of C_{17}-oxygen function, 1,2-shift of C_{18}-methyl group, and hydride shifts occur succeedingly in the earlier period of Kober reaction and that the carbocation (III) is produced, which shows a maximum absorption at 372 nm and can then be oxidized into the characteristic Kober chromophore.[1] The present paper deals with the structure elucidation of the products in the reaction of some estrane derivatives with concentrated sulfuric acid, supporting that the chemical species responsible for the maximum absorption at 372 nm is the carbocation (III).

Results and Discussion

In the preceding paper,[1] it was reported that the maximum absorption at 372 nm (ε: 33000—34000) that was stable for several hours at room temperature appeared immediately when 3-methoxy-17α-methylestra-1,3,5(10)-trien-17β-ol (IV) was dissolved in 97.2% sulfuric acid. The same absorption was observed in the cases of 3-methoxyestra-1,3,5(10)-trien-17α-ol (V), 3-methoxyestra-1,3,5(10),16-tetraene (VI), and 3-methoxy-17β-methyl-18-norestra-1,3,5-(10),13-tetraene (VII).[1] It was also reported that the nuclear magnetic resonance (NMR) spectra of IV, V, VI, and VII in concentrated sulfuric acid seemed to indicate the chemical species χ-372 showing maximum absorption at 372 nm to be the carbocations (IIIa, b).[1] The conjugate bases, Δ^8-olefins, of the cations were isolated in the present study from the reaction mixtures of these estrane derivatives with concentrated sulfuric acid, as stated below.



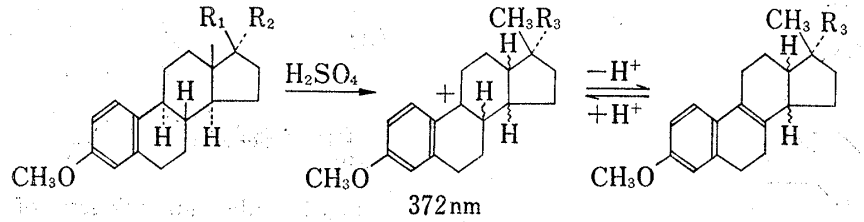
I: R_1,R_2=O
II: R_1=OH, R_2=H
IV: R_1=OH, R_2=CH_3
V: R_1=H, R_2=OH
VI: R_1=H, Δ^{16}
VII: R_3=H
IX: R_3=CH_3

Chart 1

Colorless leaflets, mp 122—124°, were obtained in 90% yield from the reaction mixture of IV with 97.2% sulfuric acid. Since mass spectrum (MS) showed the molecular ion of 282 mass unit and no signals attributed to vinyl proton were observable in NMR spectrum, the crystals thus obtained seemed to be the dehydrated compound having a tetrasubstituted double bond. Ultraviolet (UV) spectrum gave the maximum absorption at 273 nm (ε 17300) which is characteristic of *p*-methoxystyrene[10] and is immediately shifted to 372 nm on addition of concentrated sulfuric acid. NMR spectrum showed a singlet signal due to two methyl groups. It may, therefore, be reasonable to postulate that the product is the Δ^8-olefin, 3-methoxy-17,17-dimethyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VIIIb), the conjugate base of the carbocation (IIIb). When methanolic solution of IV was refluxed with 6N hydrochloric acid, Δ^{13}-olefin, 3-methoxy-17,17-dimethyl-18-norestra-1,3,5(10)-13-tetraene (IX), was obtained,[11] from which Kirdani[12] failed to isomerize into the corresponding Δ^8-olefin (VIIIb). However, the characteristic absorption at 372 nm due to the formation of carbocation (IIIb) appeared immediately when the olefin (IX) was dissolved in concentrated sulfuric acid and its isomer (VIIIb) was then isolated quantitatively. Similarly, the reactions of V—VII with 97.2% sulfuric acid at room temperature for twenty minutes gave the conjugate base (VIIIa) of carbocation (IIIa) in 80—90% yields. These dehydration and transfer reactions seemed to

9) M. Kimura, M. Kawata, K. Akiyama, K. Harita, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **21**, 1741 (1973).
10) J.E. Cole, Jr., W.S. Johson, P.A. Robins, and J. Walker, *J. Chem. Soc.*, **1962**, 244.
11) J. Torreilles and A.C. de Paulet, *Bull. Soc. Chim. France*, **1968**, 4886.
12) R. Kirdani, R.I. Dorfman, and W.R. Nes, *Steroids*, **1**, 219 (1963).

proceed instantaneously, since the absorption at 372 nm was observed after several seconds when IV—VII were dissolved in concentrated sulfuric acid[1] and the conjugate bases (VIIIa, b) of the carbocations (IIIa, b) were then obtained quantitatively from these reaction mixtures. It may be concluded from these results that the chemical species χ-372 formed initially in the Kober reaction is the carbocation (III). This type of reaction in steroids with acid has been known to proceed generally in a concerted mechanism[13] as illustrated by the backbone rear-



IV : R_1=OH, R_2=CH_3
V : R_1=H, R_2=OH

IIIa : R_3=H
IIIb : R_3=CH_3

VIIIa : R_3=H
VIIIb : R_3=CH_3

Chart 2

rangement of cholestane derivatives.[14] Since the 17α-ol (V) has a *trans-anti-trans* geometry of departing and moving group, its dehydration and transfer reactions giving the carbocation (IIIa) may also proceed in the same mechanism. The studies on the absolute configuration of IIIa are in progress now.

The epimer (II) of V showed, contrary to IV—VII, the faster spectral change as reported previously,[1] when it was dissolved in concentrated sulfuric acid. The acid solution which was allowed to stand for twenty minutes after dissolving II was poured into a large quantity of water, followed by extracting with benzene and chloroform. The extracted products, obtained in about 20% yield, seemed to be composed of three or more species which were hardly separated and their gas-liquid chromatography (GLC) showed the formation of Δ^8-olefin (VIIIa) in 5—10% yields. The remainder of II was, on the other hand, converted into the hydrophilic compound(s) which seemed to be formed through sulfonation.[15] When estrone-3-methyl ether (I) was similarly treated with concentrated sulfuric acid for twenty minutes at room temperature, formation of VIIIa was not observed and the hydrophilic product was obtained, which was then methylated to give colorless needles, mp 195—197°, $C_{20}H_{26}O_5S$, in about 70% yield. Since NMR spectrum showed no signal of C_2–H but those of aromatic protons (δ, 7.82, singlet: C_1–H and 6.78, singlet: C_4–H) as well as that due to C_2–SO_3CH_3 (δ, 3.94, singlet), the chemical structure of the methylated product may reasonably be elucidated as methyl 3-methoxyestra-1,3,5(10)-trien-17-one-2-sulfonate (X). Formation of the dienone (XI) was not observed in the reaction of I with concentrated sulfuric acid, though Jacquesy, *et al.*[16] obtained it in about 70% yield from the reaction mixture of I and HF-SbF_5. The extremely high acidity is, as they pointed out, necessary to form XI and concentrated sulfuric acid seems to be inadequately acidic for this purpose.

Numbers of organic compound undergo the complicated reactions involving protonation, dehydration, transformation, sulfonation, oxidation, and polymerization, when they come into contact with concentrated sulfuric acid. The reaction of IV or V, on the other hand, seemed to proceed so simply that Δ^8-olefin (VIIIa or b) was isolated in a high yield. This may be explained as follows: 1) The dehydration takes place more readily in the tertiary alcohol (IV) or in the secondary one (V) having *trans*-diaxial configuration of C_{17}–OH to C_{18}–

13) N.S. Wendler, "Molecular Rearrangements," Part. II, ed. by Paul de Mayo, "Rearrangements in Steroids," Interscience, Publishers, Inc., New York, 1964, p. 1019, Chap. 16.
14) D.N. Kirk and M.P. Hartshorn, "Steroid Reaction Mechanisms," Elsevier, Amsterdam, 1968, p. 261, 292.
15) Details will be reported later.
16) J.P. Gesson, J.C. Jaoguesy, and R. Jacquesy, *Tetrahedron Letters*, **1971**, 4733.

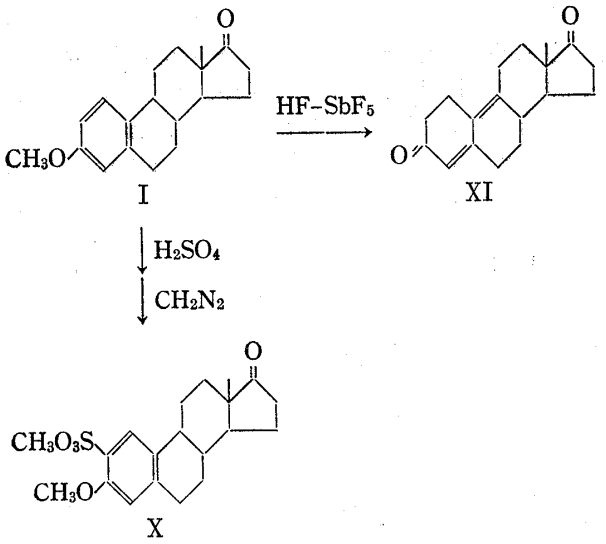


Chart 3

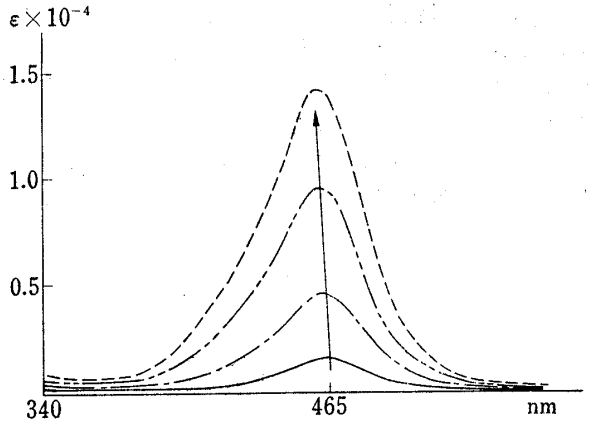


Fig. 1. Absorption Spectra of Estrone Methyl Ether (I) in 97.2% H_2SO_4

(I): 137 μg H_2SO_4: 10 ml at 25°
———: 10 min ———: 60 min
———: 30 min ———: 180 min

CH_3 than in the *cis*-isomer (II)[17,18] and the carbocation (IIIa or b) can thus be produced rapidly, which is stable in concentrated sulfuric acid and 2) The sulfonation at C_2 in the cation (III) can hardly occur due to the reduced electron density of the ring A. In fact, no change was observable in the NMR spectrum and the high yield of Δ^8-olefin (VIII) after elapse of five hours when IV—VII came into contact with concentrated sulfuric acid.

The concentrated sulfuric acid solution of I gave no absorption at 372 nm but gradually the one at 465 nm (Fig. 1)[19] which is observable at the first stage of Kober reaction and may indicate the formation of the chemical species χ-465. Since the C_2-sulfonated compound (X) was the main product from I as described above, a mere portion of the substrate (I) seems to produce χ-465 in concentrated sulfuric acid. When the solutions of IV—VII in the same acid were diluted with water, on the other hand, the absorption maximum shifted gradually from 372 to 465 nm and the oxidizing agent such as selenic acid accelerated the shift.[20] The 17β-ol (II) showed also these absorption maxima which shifted rather rapidly in a complicated fashion.[1] These facts may give a clue to the mechanism of Kober reaction and studies on the chemical structure of χ-465 are now in progress.

Experimental[21]

3-Methoxy-17,17-dimethyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VIIIb)——To a dry sample of IV (410 mg) was added conc. H_2SO_4 (15 ml) with vigorous stirring. The reaction mixture became homogeneous and slightly yellow with green fluorescene. The stirring was continued further for 20 min at room temperature, and the colored solution was dropped into vigorouly stirred ice water (300 ml) and then extracted with benzene. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to dryness yielding a

17) T. Lunaas, *Acta Chem. Scand.*, **18**, 321 (1964).
18) D.N. Kirk and M.P. Hartshorn, "Steroid Reaction Mechanisms," Elsevier, Amsterdam, 1968, p. 269.
19) The maximum at 465 nm shifted to 452 nm with the elapse of time. This is due to the sulfonation at C_2 and will be discussed in a subsequent paper.
20) Details will be reported in the near future.
21) Melting points were taken on a micro hot-stage apparatus and are uncorrected. UV and IR spectral measurements were run on Hitachi Model 3T recording and JASCO Model IR-S spectrometers, respectively. NMR spectra were obtained by Hitachi Model R-20-B spectrometer at 60 Mc in $CDCl_3$ using tetramethylsilane as an internal standard. Mass spectra were measured by Hitachi Model RMU-6R spectrometer. GLC was run on Shimadzu Model GC-4APF gas chromatograph using a stainless steel column (3 m×3 mm i.d.) of 1.5% SE-30 on Shimalite W (60—80 mesh) with a N_2 flow of 60 ml/min (2.0 kg/cm²) and a hydrogen flame ionization detector; the temperatures of column, detector, and injection chamber were kept at 200°, 250°, and 220°, respectively. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Abbreviation used s=singlet, d=doublet, t=triplet, and m=multiplet.

crystalline residue (381 mg), recrystallization (EtOH) of which gave colorless leaflets, mp 122—124°. *Anal.* Calcd. for $C_{20}H_{26}O$: C, 85.08; H, 9.28. Found: C, 84.91; H, 9.46. Mass Spectrum *m/e*: 282 (M^+). NMR (δ in $CDCl_3$): 1.02 (6H, s, C_{17}-gem-CH_3), 3.77 (3H, s, OCH_3), 6.65—7.10 (3H, arom.). UV λ_{max}^{EtOH}: 273 nm (ε=17300). $[\alpha]_D^{20}$: −9.2° (c=1.25, $CHCl_3$).

3-Methoxy-17β-methyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VIIIa)——To a dry sample of VI (215 mg) was added conc. H_2SO_4 (10 ml) with vigorous stirring. The resulting pale yellow solution was stirred further for 20 min at room temperature and then dropped into vigorously stirred ice water (300 ml). The solution thus obtained turned slightly pink and was extracted with benzene. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to dryness, yielding a colorless oil (198 mg). *Anal.* Calcd. for $C_{19}H_{24}O$: C, 85.02; H, 9.01. Found: C, 84.59; H, 9.00. Mass Spectrum *m/e*: 268 (M^+). NMR (δ in $CDCl_3$): 1.00 (3H, d, J=7 cps), 3.71 (3H, s, OCH_3), 6.65—7.10 (3H, arom.). UV λ_{max}^{MeOH}: 273 nm (15900). $[\alpha]_D^{20}$: +38.7° (c=1.24, $CHCl_3$). Attempts to crystallize the colorless oil was in no success. Gas chromatography (1.5% SE-30, 200°, t_R=8.5 min) and UV indicated that this material was of about 90% purity. Under the same condition, VIIIa was obtained in 80—90% yield from V or VII.

Isomerization of IX to VIIIb——The mixture of IX (98 mg) and conc. H_2SO_4 (7 ml) was stirred for 3 min at room temperature and was then dropped into vigorously stirred ice water (200 ml). The reaction mixture was extracted with benzene and the organic layer was washed with water, dried over Na_2SO_4 and evaporated to dryness, yielding a crystalline residue (89 mg). Recrystallization of the residue from EtOH yielded 3-methoxy-17,17-dimethyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VIIIb) as colorless leaflets, mp. 122—124°.

Methyl 3-Methoxyestra-1,3,5(10)-trien-17-one-2-sulfonate (X)——The mixture of I (286 mg) and conc. H_2SO_4 (15 ml) was stirred at room temperature. The solution became yellow with green fluorescene as I was dissolved. After stirring for 20 min at room temperature, the solution was dropped into vigorously stirred ice water (300 ml) which turned to pink. No product was extracted with benzene or chloroform from the pink solution. The pink solution was submitted to chromatography on amberlite XAD-2 column. Evaporation of the solvent from the first fraction eluted with MeOH (4l) left a residue which was then methylated with CH_2N_2, giving a crystalline residue (246 mg). Recrystallization of the residue yielded colorless needles, mp 195—197°, from MeOH. *Anal.* Calcd. for $C_{20}H_{26}O_5S$: C, 63.48; H, 6.93; S, 8.46. Found: C, 63.26; H, 6.91; S, 8.58. Mass Spectrum *m/e*: 378 (M^+). NMR (δ, in $CDCl_3$); 0.94 (3H, s, CH_3) 3.82 (3H, s, OCH_3) 3.94 (3H, s, SO_3CH_3), 6.78 (1H, s, C_4-H), 7.82 (1H, s, C_1-H). IR spectrum ν_{max}^{Nujol} cm^{-1}: 1733 (>C=O), 1350, 1170 (-SO_2-).