[Chem. Pharm. Bull. 21(8)1741—1746(1973)]

UDC 547.92.04 : 546.226.35.04

Chromogenic Reactions of Steroids with Strong Acids. V.[1] Products in the Kober Reaction of Steroidal Estrogens[2]

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(Received January 16, 1973)

Formation and chemical structures of some products were studied in the Kober color reactions of estradiol 3-methyl ether (I) and of estrone methyl ether (II). After heating I or II with 78% (w/w) sulfuric acid at 100° for about half-hour, extraction of the reaction-mixture with benzene left the Kober-pigment in the acidic layer, which was collected in a reddish purple regin and was instable in a neutral or alkaline aqueous solution. In this reaction, were produced 3-methoxy-17ξ-methyl-8ξ,9ξ,13ξ,14ξ-18-norestra-1,3,5(10)-triene (III), 3′-methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene (IV), 3′-methyl-7-methoxy-1,2-cyclopentenophenanthrene (V), 3-methoxy-17ξ-methyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VI), and 3-methoxy-17ξ-methyl-13ξ,14ξ-18-norestra-1,3,5(10),6,8-pentaene (VII) from I. The same reaction of II gave these compounds (III—VII) and the new products, methyl 3′-methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene-6-sulfonate (VIII) and 3′-methyl-7-methoxy-1,2-cyclopentadieno-9,10-dihydrophenanthrene (IX).

In the preceding paper[1] of this series, the specificity of the Kober reaction was studied and it was suggested that a compound will give a pink color reaction when the following features are wholly present in a molecule: (1) steroidal ring system, (2) aromatic ring A with an oxygen function at C_3, (3) oxygen function at C_{17} and/or C_{16} except the diketo function at both positions, (4) angular methyl group at C_{13}, and (5) angular hydrogen atom. Boscott[4] postulated that the initial step of the Kober reaction involves dehydration at the oxygenated site in ring D. Kägi, *et al.*,[5] Boscott,[4] and Jones, *et al.*[6] suggested that the angular methyl migration from C_{13} to C_{17} occurred concomitant with the dehydration. The initial formation of the carbocation and its subsequent transformation to the pink chromophore were observed in this reaction of several phenolic steroids.[2b] In spite of numerous studies on the colorimetric procedures and spectroscopic observations, little has ever been reported on the detailed survey of the products formed in the Kober reaction. The main purpose of the present research was directed to the isolation and the structure elucidation of these products, which may be expected to provide some valuable informations on the chemical structure (s) of the characteristic chromophore (s) and on the mechanism of the Kober reaction. In this paper, the formation of several steroidal olefins will be described, which were produced in the reactions of methylated estradiol (3-methoxyestra-1,3,5(10)-trien-17β-ol) (I) and of methylated estrone (3-methoxyestra-1,3,5(10)-trien-17-one) (II) with hot and moderately concentrated sulfuric acid.

1) Part IV: M. Kimura, M. Kawata, K. Akiyama, K. Harita, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **21**, 1720 (1973).
2) Preliminary accounts of a part of this work have been published: *a*) M. Kimura, K. Akiyama, K. Harita, T. Miura, and M. Kawata, *Tetrahedron Letters*, **1970**, 377; *b*) 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) R.J. Boscott, *Nature*, **164**, 140 (1949).
5) H. Kägi and K. Miescher, *Helv. Chim. Acta*, **22**, 683 (1939).
6) H.A. Jones and R. Hähnel, *Steroids*, **13**, 693 (1969).

Result and Discussion

Formation and Properties of the Kober Chromophore

The Kober test is a two phase reaction. The first is heating at 100° for about twenty minutes with 50—80% (v/v) sulfuric acid, which produces a yellow solution, and the second phase is dilution with water or diluted sulfuric acid and reheating, which produces a pink colored solution. Although various modified Kober reactions have been employed for the colorimetry by using sulfuric acid with some additional reagents such as phenols[7] and hydroquinone,[8] each of the steroidal estrogens examined gave almost same chromatogram in the thin-layer chromatography (TLC) (BuOH–HAc–water=2:1:1 on Wakogel B-5) of the contents of their respective reaction-mixtures, whenever the substrate was treated with these modified reagents or merely with sulfuric acid excluding such additives. Extraction of the reaction-mixture with benzene gave an organic layer showing characteristic fluorescence and left the Kober-pigment in the acidic layer, which was collected in a reddish purple reginous state by centrifugation. Similar chromatograms in TLC (*n*-hexane: benzene=1:1 on Wakogel B-5) of the benzene-extracts and also in the paper electrophoresis (pH 4.3, 0.05M NH_4OAc–HAc buffer, 700 V, 2.3 mA, 1 hr) of the resinous pigment (Fig. 1) were similarly observable in the reactions which were carried out with the identical substrate as differently as mentioned above. Thus, the reaction of estradiol 3-methyl ether (I) or estrone 3-methyl ether (II) merely with hot 66% (v/v) or 78% (w/w) sulfuric acid without any additives seemed to be profitable for the purpose of the survey of the products.

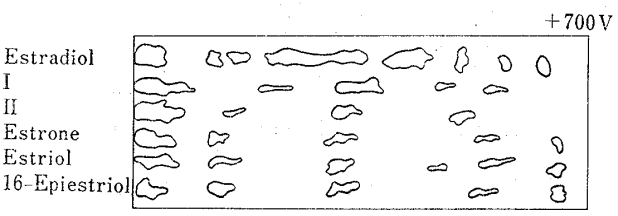


Fig. 1. Paper Electrophoresis of the Kober-Pigment

pH 4.3 (0.05M NH_4OAc–HAc buffer), 700 V, 2.3 mA, 1 hr
Spots were detected by using UV-lamp.

When the Kober reaction of I or II was carried out in such a preparative way, the pigment was found to be produced even early at the first phase of the reaction. Contrary to be instable in a neutral and particularly in an alkaline aqueous solution, the pigment was rather stable giving the visible light absorption similar to that shown in the practice of the colorimetry using the Kober reaction when it was dissolved in the same concentration of sulfuric acid with that initially employed for its formation. Although the Kober-pigment thus formed cannot be transfered to the non-polar organic solvent such as benzene as described above, Ittrich[9] found the mixture of chloroform and ethylalcohol (99:1; v/v) containing *p*-nitrophenol (2%) to be an appropriate one, that led the sensitive and selective method for the spectroscopic determination of estrogens in the biological samples. From the column chromatography of the benzene-extracts over silica gel, on the other hand, the dehydrogenated derivatives were obtained as described below. The formation of sulfur dioxide and hydrogen sulfide was also observed during course of the Kober reaction. These facts are likely to reveal that some oxidation processes are involved in this reaction.

Products from Estradiol 3-Methyl Ether (I)

The substrate (I) was heated at 100° for twenty minutes with 78% (w/w) sulfuric acid under stirring, during which time the formation of sulfur dioxide and hydrogen sulfide was

7) L.R. Axelrod, *J. Biol. Chem.*, **201**, 59 (1953); H.W. Marlow, *ibid.*, **183**, 167 (1950).
8) J.B. Brown, *Biochem. J.*, **60**, 185 (1955); A. Kambegawa, *Nippon Naibumpi Gakkai Zasshi*, **37**, 807 (1961) [*C. A.*, **56**, 9352b (1962)].
9) G. Ittrich, *Z. Physiol. Chem.*, **312**, 1 (1958); *idem*, *Acta Endocrinol.*, **35**, 34 (1960).

observable. The chromatography of the benzene-extracts from the reaction-mixture gave two kinds of colorless oils which were stable for sulfuric acid, had common formula of $C_{19}H_{26}O$ (M^+: 270), and were similar in TLC and infrared (IR) as well as ultraviolet (UV) (anisole type) spectra but different in gas–liquid chromatography (GLC). Although formation of dehydrated products might readily be expected, none of these oils came into reaction with osmic acid and gave the different retention times (t_R=7.47 and 8.97) in GLC from that (t_R=9.90) due to 3-methoxyestra-1,3,5(10)-triene which gave similar mass spectrum (MS) to those of them. From these results and the nuclear magnetic resonance (NMR) spectral data showing the doublet methyl signals at the region of δ 1 (J=7 cps), it may be concluded that these oils have commonly the structure of 3-methoxy-17ξ-methyl-8ξ,9ξ,13ξ,14ξ-18-norestra-1,3,5(10)-triene (III). Since both of them were nearly inactive in optical rotation, these colorless oils might be assumed to be isomeric mixtures, in spite of that each of them gave a single peak in GLC.

The chromatography of the benzene-extracts mentioned above gave another product as colorless flakes, mp 83°, $C_{19}H_{20}O$, M^+ 264. Its ultraviolet absorption spectrum kept the biphenyl type showing maximum at 282 nm (log ε=4.41). In NMR spectrum there appeared five aromatic protons at δ 6.7—7.8, methyl signals at δ 1.30 (doublet, J=7 Hz), and four benzylic protons at δ 2.8 in a narrow range as commonly shown by the dihydrophenanthrenes. The chemical structure of this product may thus be elucidated to be 3′-methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene (IV). Colorless prisms (V), mp 149°, were also obtained through the same chromatography. Ultraviolet absorption spectrum of V was of the phenanthrene type and the signals of OCH_3 as well as CH_3 in NMR shifted towards lower field than those of IV. When V was treated in isoamyl alcohol with metallic sodium, the dihydrophenanthrene (IV) was formed, which was dehydrogenated back to V with selenium. The chemical structure of V may thus be elucidated to be 3′-methyl-7-methoxy-1,2-cyclopentenophenanthrene, which was identified further with the authentic specimen.[10] The other fractions in the above-mentioned chromatography gave small amounts of the olefins, 3-methoxy-17ξ-methyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VI) and 3-methoxy-17ξ-methyl-13ξ,14ξ-18-norestra-1,3,5(10),6,8-pentaenes (VII), the latter of which showed UV spectrum of the naphthol type; it was unfortunate that their further purification resulted in no success.

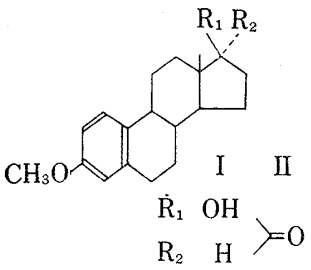
Products from Estrone Methyl Ether (II)

The methyl ether (II) was similarly heated at 100° for forty minutes with 78% (w/w) sulfuric acid and the reddish purple resinous products were collected through centrifugation, which were then methylated with diazomethane and submitted to chromatography on silica gel. Chloroform-extracts from the diluted supernatant, on the other hand, were also submitted to chromatography, after recovery of about one third in amount of the substrate (II). The same products (III—VII) of I and the following new products (VIII and IX) were thus obtained through these chromatographic procedures.

From the methylated resine, colorless prisms (VIII), mp 143.5°, $C_{20}H_{22}O_4S$, M^+ 356, were obtained, which showed UV absorption at 282.5 nm (log ε=4.40) similarly to that of dihydrophenanthrene (IV) and gave characteristic IR spectrum, 1396 and 1184 cm^{-1}, indicating the presence of sulfonate function. In NMR spectrum of VIII, there appeared several peaks at δ 8.18 (singlet), 7.55 (doublet), 7.08 (doublet), and 6.85 (singlet). Since these two kinds of single peak may be assumed to be due to the protons at C_1 and C_4, it is possible to conclude that the sulfonation occurred at C_2 in the steroidal ring A. Thus, the structure of this product was elucidated to be methyl 3′-methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene-6-sulfonate (VIII). From the chromatography of the supernatant, on the other hand, were obtained colorless needles, mp 214—215.5°, $C_{19}H_{18}O$, M^+ 262 (IX).

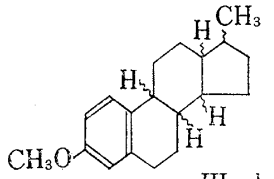
10) A. Cohen, J.W. Cook, and C.L. Hewett, *J. Chem. Soc.*, **1935**, 445.

Ultraviolet spectrum of this product gave absorption maxima at 215, 308, and 326 (shoulder) nm and showed the same pattern with those of IV as well as VIII, shifting in the more bathochromic region and thus indicating the prolongation of the conjugated system present in these olefinic compounds. It may, therefore, be concluded that this olefin has the chemical structure of 3′-methyl-7-methoxy-1,2-cyclopentadieno-9,10-dihydrophenanthrene (IX). Although TLC and GLC of this product were both indicative of its unity, NMR signals due to 3′-CH_3 appeared doubly at δ 1.58 (doublet, J=8 Hz) and 1.76 (singlet) and it may be pointed out from their integral intensities that the product (IX) is the isomeric mixture of 3′-methyl-7-methoxy-1,2-cyclopenta-4′-dieno-9,10-dihydrophenanthrene (IXa) and the 3′-dieno compound (IXb) in a ratio of 5: 3.

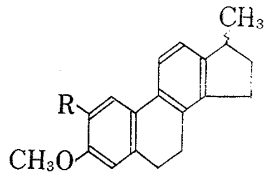


I II

R_1 OH
R_2 H >=O

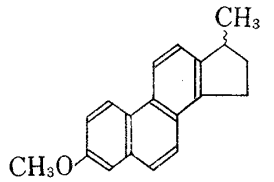


IIIa, b

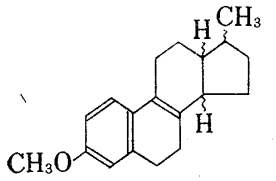


IV VIII

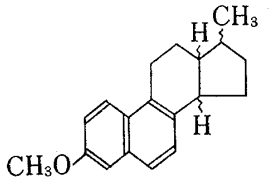
R H SO_3CH_3



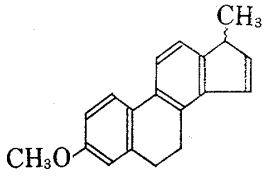
V



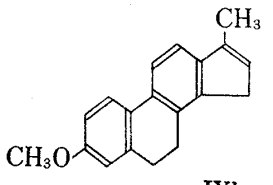
VI



VII



IXa



IXb

Experimental[11]

Materials——Estradiol (estra-1,3,5(10)-triene-3,17β-diol), estrone (3-hydroxyestra-1,3,5(10)-trien-17-one), estriol (estra-1,3,5(10)-triene-3,16α,17β-triol), and 16-epiestriol (estra-1,3,5(10)-triene-3,16β,17β-triol) were obtained from the commercially available sources and the methyl ethers (I and II) as well as 3-methoxyestra-1,3,5(10)-triene were derived from the respective substrate thus obtained. These materials were purified by the ordinary method.

Formation and Isolation of the Products from Estradiol Methyl Ether (I)——A mixture of I (6.19 g) and 78% (w/w) H_2SO_4 (300 ml) was heated for 20 min in a boiling water-bath under stirring. Formation of sulfur dioxide and hydrogen sulfide was observed by decoloring the iodine solution and blackening the lead acetate solution, respectively. After the reddish purple reaction-mixture was cooled with running water, it was poured into water (1000 ml) and was extracted with benzene (1000 ml×3). The organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to give a brown oil (3.38 g) which was then submitted to chromatography on silica gel (100 g) and eluted with *n*-hexane and *n*-hexane–benzene (8: 2). The effluents with *n*-hexane were pooled into three fractions (1—3) under the indication of gas chromatographic data. Similarly, five fractions (4—8) were obtained from the effluents with hexane–benzene.

11) 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 H-6013 spectrometer at 60 Mc in $CDCl_3$ using tetramethylsilane as an internal standard. Mass spectra were measured by Hitachi Model RMU-6E 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 44 ml/min (1.0 kg/cm^2) and a hydrogen flame ionization detector; the temperatures of column, detector, and injection chamber were kept at 185°, 220°, and 240°, respectively. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Abbreviation used s=singlet, d=doublet, t=triplet, and m=multiplet.

3-Methoxy-17ξ-methyl-8ξ,9ξ,13ξ,14ξ-18-norestra-1,3,5(10)-trienes (IIIa and IIIb)——The fraction 1 (15 mg in contents) eluted with *n*-hexane as described above was found to involve IIIa as a major component. A portion (980 mg) of the fraction 2 (1380 mg in contents) was submitted again to chromatography in a same way to give colorless oils, IIIa (80 mg) and IIIb (25 mg).

IIIa: Colorless oil. *Anal.* Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.21; H, 9.70. $[\alpha]_D^{16}=0°$ ($c=1.86$). UV $\lambda_{max}^{isooctane}$ nm(log ε): 221 (3.60), 278 (3.34), 287 (3.39) (anisole type). IR ν_{max}^{direct} cm^{-1}: 1610, 1570, 1500, 865, 835 (arom.). NMR δ ppm: 7.13 (1H, d, $J=9$ Hz, C_1–H), 6.53 (1H, dd, $J=9$, 3 Hz, C_2–H), 6.46 (1H, s, C_4–H), 3.75 (3H, s, OCH_3), 0.82 (3H, d, $J=7$ Hz, CH_3). Mass Spectrum *m/e*: 270 (M^+). GLC: $t_R=7.47$.

IIIb: Colorless oil. *Anal.* Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.43; H, 9.60. $[\alpha]_D^{26.5}=0°$ ($c=1.02$). GLC: $t_R=8.97$. Other physical data were entirely (UV and MS) or almost (IR and NMR) identical with those of IIIa.

To an ether solution (30 ml) of the mixture (472 mg) of IIIa and IIIb, was added a pyridine solution (0.8 mg) of OsO_4 (611 mg) and the reaction-mixture was stirred for 4.5 days at room temperature. The solution was then refluxed for 70 min with the mixture of water (10 ml), EtOH (20 ml), and Na_2SO_3 (0.5 g). After adding 300 ml of water, the solution was extracted with benzene. The organic layer was treated in an ordinary way and the intact substrate (457 mg) was recovered, which gave the same gas chromatogram with that due to the mixture of IIIa and IIIb. Inertness of the substrate was also observed in the reaction of the mixture of IIIa and IIIb with hot 78% (w/w) H_2SO_4.

3′-Methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene (IV)——The contents (663 mg) of the fraction 5 mentioned above were recrystallized from MeOH–benzene to give colorless flakes, mp 82—83°. *Anal.* Calcd. for $C_{19}H_{20}O$: C, 86.32; H, 7.63. Found: C, 86.49; H, 7.84. $[\alpha]_D^{22}=0°$ ($c=1.31$). UV $\lambda_{max}^{isooctane}$ nm(log ε): 282 (4.41) (biphenyl type). IR ν_{max}^{KBr} cm^{-1}: 1618, 1590, 1570, 1502, 877, 810 (arom.). NMR δ ppm: 7.8—6.7 (5H, arom.), 3.80 (3H, s, OCH_3), 2.80 (4H, m, $C_{9,10}$–H), 1.30 (3H, d, $J=7$ Hz, CH_3). Mass Spectrum *m/e*: 264 (M^+). GLC: $t_R=20.0$.

3′-Methyl-7-methoxy-1,2-cyclopentenophenanthrene (V)——The contents (206 mg) of the fraction 7 mentioned above were recrystallized from MeOH to give colorless prisms, mp 147—149°. *Anal.* Calcd. for $C_{19}H_{18}O$: C, 86.98; H, 6.91. Found: C, 86.81; H, 6.97. $[\alpha]_D^{21}=0°$ ($c=1.54$). UV $\lambda_{max}^{isooctane}$ nm(log ε): 225 (4.15), 237 (4.17), 255 (4.35), 262 (4.50), 283 (4.15), 292 (4.14), 302 (4.13), 326 (2.93), 341 (3.14), 358 (3.17) (phenanthrene type). IR ν_{max}^{KBr} cm^{-1}: 1615, 1580, 1530, 855, 810, 800 (arom.). NMR δ ppm: 8—7 (7H, arom.), 4.00 (3H, s, OCH_3), 1.40 (3H, d, $J=7$ Hz, CH_3). Mass Spectrum *m/e*: 262 (M^+). GLC: $t_R=36.0$. Melting point and IR spectrum were identical with those of the authentic specimen prepared by the method of Cohen, *et al.*[10]

Dehydrogenation of IV: In a quartz tube, the mixture of IV (53 mg) and Se powder (200 mg) was heated at 350° for 15 min. EtOH extracts of the products were submitted to preparative TLC to give crude crystalls (32 mg). The addition compound (45 mg), which melted at 136° and without depression in admixture with the authentic sample, was obtained by recrystallizing the mixture of the crude crystalls (27 mg) and 1,3,5-trinitrobenzene (27 mg) from EtOH. After $SnCl_2$ solution was added dropwise to a EtOH solution of this addition compound (40 mg), the reaction-mixture was diluted with water and extracted with ether. The organic layer was treated in an ordinary way to give V (15 mg) which was identical in melting point and IR spectrum with those of the authentic specimen.

Reduction of V: After metallic Na (12.4 g) was added gradually to the boiling isoamylalcoholic solution (200 ml) of V (116 mg), the solution was refluxed further for 1 hr and then cooled with running water. The solution was acidified with HCl and the organic layer was washed with water. The aqueous layer was extracted with benzene. The organic layers thus obtained were combined and the solvent was evaporated *in vacuo* to leave a pale-yellow oil (110 mg). Chromatography of this oil on silica gel gave colorless flakes (80 mg), mp 82° (MeOH), which showed no depression of melting point in admixture with the authentic specimen of IV.

3-Methoxy-17ξ-methyl-13ξ,14ξ-18-norestra-1,3,5(10),8-tetraene (VI)——The contents (210 mg) of the fraction 3 mentioned above were purified through chromatography to give a colorless oil. UV λ_{max}^{EtOH}: 275 nm. Mass Spectrum *m/e*: 268 (M^+). GLC: $t_R=11.3$. The oil gave the identical peak in GLC with that of the authentic specimen of VI which was derived from the isomeric olefin (3-methoxy-17β-methyl-18-norestra-1,3,5(10),13-tetraene)[12] on treatment with formic acid as follows:

Preparation of the Authentic Specimen: The mixture of this isomeric tetraene (225 mg) and anhydrous formic acid (300 ml) warmed at 40—50° was filtered with glass-filter and the filtrate was kept at 40° for 1 hr. Evaporation of formic acid *in vacuo* at 40° from the filtrate left a pale-yellow oil which was then submitted to chromatography on silica gel (10 g). The column was developed with *n*-hexane and a colorless oil (201 mg) (VI) was obtained, besides IIIa and/or IIIb (7 mg) and IV (4 mg). *Anal.* Calcd. for $C_{19}H_{24}O$: C, 85.02; H, 9.01. Found: C, 85.27; H, 8.91. UV λ_{max}^{EtOH} nm(log ε): 273 (4.19) (characteristic of *p*-methoxystyrene). IR ν_{max}^{direct} cm^{-1}: 1610, 1580, 885, 810, 780 (arom.). NMR δ ppm: 7.5—6.5 (3H, arom), 3.76 (3H, s, OCH_3), 0.84 (3H, d, $J=7$ Hz, CH_3). Mass Spectrum *m/e*: 268 (M^+). GLC: $t_R=11.3$.

12) W.F. Johns, *J. Org. Chem.*, **26**, 4583 (1961).

3-Methoxy-17ξ-methyl-13ξ,14ξ-18-norestra-1,3,5(10),6,8-pentaenes (VII)——Besides VI, the above-mentioned re-chromatography of the fraction 3 gave also a colorless oil. UV $\lambda_{max}^{isooctane}$ nm: 228, 257, 266, 275, 286, 307, 315, 321, 330, 336 (naphthol type). GLC: t_R=13.4, 14.9, and 16.4.

Formation and Isolation of the Products from Estrone Methyl Ether (II)——A mixture of II (10 g) and 78% (w/w) H_2SO_4 (300 ml) was kept at 100° for 40 min, during which time the formation of sulfur dioxide was observed as in the case of I but no hydrogen sulfide was detectable. After cooling with water, the reaction-mixture was centrifuged (3000 r/min) for 10 min to collect the reddish purple resin (7 g) which was washed once with water (5 ml) and methylated at room temperature in MeOH (300 ml) with 0.2M CH_2N_2 ether solution (300 ml). The solution was allowed to stand for 24 hr and the solvent was evaporated *in vacuo* to give a concentrated solution (30 ml) which was then diluted with water (30 ml) and extracted with benzene (500 ml). Evaporation of the solvent from the organic layer left a residue (1.86 g) which was submitted to chromatography on silica gel (60 g) by developing successively with benzene, benzene–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH, and finally with MeOH. The contents (0.468 g) of the fractions eluted with benzene were purified further through chromatography and/or recrystallization and were found to be consisted mainly of IIIa, IIIb, IV, and V; they were identified by comparing their (IIIa and IIIb) IR, UV, and GLC data or their (IV and V) melting points and IR data with those of the respective authentic samples.

The supernatant obtained by centrifuging the reaction-mixture of II and H_2SO_4 described above was diluted with water (520 ml) and extracted with $CHCl_3$ (1000 ml×3). Evaporation of the solvent from the organic layer left a residue (5.82 g) which was recrystallized twice from EtOH to recover the intact substrate (4.28 g). The same reaction and subsequent procedure were carried out again with recovered substrate and the combined EtOH mother-solution thus obtained left a resinous residue (4.29 g) by evaporation of the solvent *in vacuo*, which was then submitted to chromatography on silica gel (100 g) by developing successively with *n*-hexane, *n*-hexane–benzene, benzene, benzene–MeOH, and finally with MeOH. The contents (0.125 g) of the fraction eluted with *n*-hexane were purified further through chromatography and were found to be mainly consisted of IIIa and IIIb. Similarly, IIIa, IIIb, and VII were found to be the major components in the effluents (0.752 g in contents) with *n*-hexane–benzene (9: 1). The first fraction (0.430 g in contents) and the second one (0.303 g) eluted with *n*-hexane–benzene (8: 2) were found to contain mainly IV and V, respectively. Identification of VII in these effluents was carried out by comparring t_R with those of the authentic specimens and their admixture. The other products such as IIIa, IIIb, IV, and V were identified in the same ways as described above. The intact substrate (II; 1.414 g) was recovered from the fraction eluted subsequently with benzene. Evaporation of the solvent from the fractions eluted finally with MeOH left a residue (0.923 g) which was not identified.

Methyl 3′-Methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene-6-sulfonate (VIII)——The fractions eluted with $CHCl_3$–MeOH (98: 2) in the chromatography of the methylated resin mentioned above left a residue (0.504 g) by evaporating the solvent under reduced pressure. The residue was recrystallized from EtOH to give colorless prisms, mp 143—143.5°. *Anal.* Calcd. for $C_{20}H_{22}O_4S$: C, 67.02; H, 6.19; S, 8.93. Found: C, 67.20; H, 6.31; S, 8.73. UV λ_{max}^{MeOH} nm(log ε): 282.5 (4.40) (biphenyl type). IR ν_{max}^{KBr} cm^{-1}: 1608, 1595, 1500, 883, 830, 780 (arom.), 1396, 1184 (–SO_2–). NMR δ ppm: 8.18 (1H, s, C_5–H), 7.55 (1H, d, J=8 Hz, C_4–H), 7.08 (1H, d, J=8 Hz, C_3–H), 6.85 (1H, s, C_8–H), 3.90 (3H, s, SO_3CH_3), 3.76 (3H, s, OCH_3), 2.8 (4H, m, $C_{9,10}$–CH_2–CH_2–), 1.29 (3H, d, J=7 Hz, CH_3–C_3). Mass Spectrum *m/e*: 356 (M^+).

3′-Methyl-7-methoxy-1,2-cyclopentadieno-9,10-dihydrophenanthrenes (IX)——In the chromatography of the products involved in the supernatant mentioned above, the third fraction was collected also by eluting with *n*-hexane–benzene (8: 2). Evaporation of the solvent *in vacuo* from the fraction left a residue (0.082 g) which was recrystallized from benzene–EtOH to give colorless needles, mp 214—215.5°. *Anal.* Calcd. for $C_{19}H_{18}O$: C, 86.98; H, 6.91. Found: C, 86.93; H, 7.01. UV $\lambda_{max}^{isooctane}$ nm: 215, 308, 326 (shoulder). IR ν_{max}^{KBr} cm^{-1}: 1614, 1585, 855, 840, 812, 800 (arom.). NMR δ ppm: 3.80 (3H, s, OCH_3), 2.83 (4H, s, $C_{9,10}$–H), 1.76 (3H, s, CH_3) (IXb), 1.58 (3H, d, J=8 Hz, CH_3) (IXa). Mass Spectrum *m/e*: 262 (M^+).

Acknowledgement The authors are gratefully acknowledged to the staffs of the central analytical laboratory of this Faculty for the elemental analysis, and measurements of NMR and mass spectra spectroscopies.