[Chem. Pharm. Bull. 26(11)3567—3571(1978)]

UDC 547.92.02.04 : 543.422.25.06

Synthesis of 2,3,17β-Trihydroxyestra-1,3,5(10)-trien-6-one and Its Related Compounds[1]

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(Received May 6, 1978)

2,3,17β-Trihydroxyestra-1,3,5(10)-trien-6-one (**7**) and its methylated derivatives (**6**, **12**, and **16**) have been prepared for the biological investigation. The preparation method and the results of the instrumental analyses including nuclear magnetic resonance and ultraviolet spectra of these materials are described.

Keywords——catechol estrogen; 6-oxo-estrogen; 6-oxo-catechol estrogen; nuclear magnetic resonance spectrum; UV spectrum of benzocyclanone derivatives

Catechol estrogens, the main metabolites of estradiol,[3] are strong competitive inhibitors for the methylation of catecholamines by the enzyme catechol O-methyltransferase.[4] By this enzyme, 2-hydroxyestradiol (**1**) is converted to the corresponding 2- and 3-monomethyl ethers, **9** and **13**,[5] and catecholamines are methylated as well-known to afford *m*- and *p*-methylated derivatives.[6] The mechanism of the enzymatic methylation of these hormonally active catechols, however, seems to be different between catecholamines and catechol estrogens.

In the case of catecholamines, the methylation ratio is depending upon the nature and the positions of substituents on the catechol ring.[6] In contrast with the case of catecholamines, 2-hydroxyestrogens gave isomeric mono-methylated products in almost equal amount.[5]

Catechol estrogen having a substituent at ring-B, therefore, may be an interesting compound in that which phenolic hydroxyl is more methylated by the enzyme. This communication deals with the preparation method of such steroid as 2,3,17β-trihydroxyestra-1,3,5(10)-trien-6-one(**7**) and also its mono- and dimethyl ethers for the potential metabolites.

The preparation of **7** was carried out by the following procedure. 2,3-Dimethoxy-17β-hydroxyestra-1,3,5(10)-triene acetate (**3**)[7] was treated with chromium trioxide in acetic acid by the method as described by Wintersteiner.[8] The corresponding 6-ketone (**5**), 2,3-dimethoxy-17β-hydroxyestra-1,3,5(10)-trien-6-one acetate, was obtained in the yield of 40%. Saponification of **5**, followed by demethylation with pyridine–hydrochloric acid gave the desired 2-hydroxyestradiol-6-one (**7**).

1) This paper constitutes Part V of the series entitled "Clinical Analysis on Steroids"; Part IV: A. Tashiro, H. Hashimoto, H. Tajiri, T. Miyata, A. Yamaguchi, K. Shinozaki, I. Makino, S. Nakagawa, and I. Yoshizawa, *Acta Hepatologica Japonica*, **19**, 52 (1978).
2) Location: *Katsuraoka-cho, Otaru, Hokkaido, 047, Japan.*
3) J. Fishman, R.I. Cox, and T.F. Gallagher, *Arch. Biochem. Biophys.*, **90**, 318 (1960); I. Yoshizawa and J. Fishman, *J. Clin. Endocr. Metab.*, **32**, 3 (1971).
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5) R. Knuppen and H. Breuer, *Hoppe-Seyler's Z. Physiol. Chem.*, **346**, 114 (1966); J. Fishman, M. Miyazaki, and I. Yoshizawa, *J. Am. Chem. Soc.*, **89**, 7147 (1967); M. Miyazaki, I. Yoshizawa, and J. Fishman, *Biochemistry* (Washington), **8**, 1669 (1969).
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7) I. Yoshizawa, M. Tamura, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **20**, 1842 (1972).
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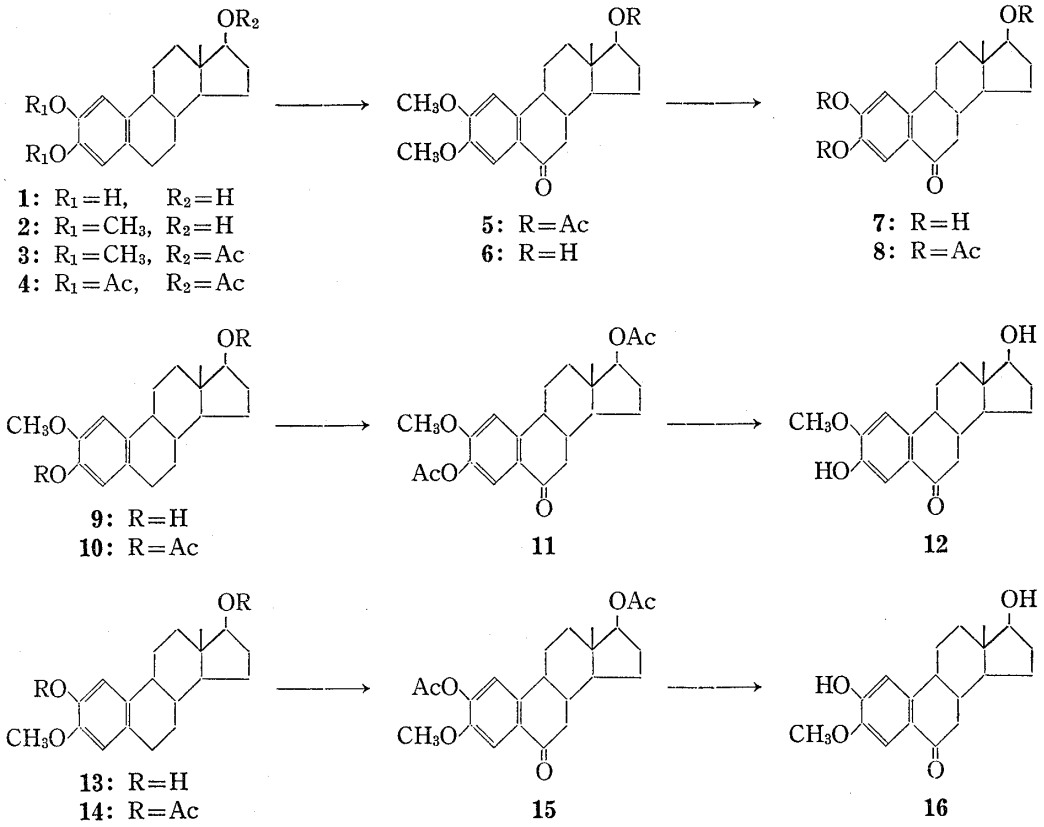
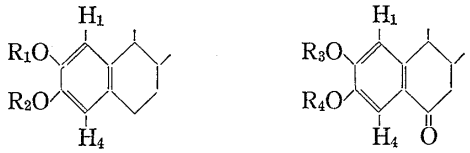


Chart 1

TABLE I. Chemical Shifts (δ in ppm) of Aromatic Protons in 2,3-Disubstituted Estra-1,3,5(10)-trien-17β-ol and Their 6-Oxo Derivatives



Compound	R_1	R_2	R_3	R_4	H_1	H_4	17β	Solvent
3	CH_3	CH_3	—	—	6.80	6.58	AcO	$CDCl_3$
5	—	—	CH_3	CH_3	6.88	7.56	AcO	$CDCl_3$
2[a]	CH_3	CH_3	—	—	6.83	6.60	OH	$CDCl_3$
6	—	—	CH_3	CH_3	6.86	7.53	OH	$CDCl_3$
10	CH_3	Ac	—	—	6.90	6.74	AcO	$CDCl_3$
11	—	—	CH_3	Ac	6.93	7.73	AcO	$CDCl_3$
9[a]	CH_3	H	—	—	6.80	6.48	OH	MeOH-d_4
12	—	—	CH_3	H	6.95	7.40	OH	MeOH-d_4
14	Ac	CH_3	—	—	6.97	6.68	AcO	$CDCl_3$
15	—	—	Ac	CH_3	7.10	7.66	AcO	$CDCl_3$
13[a]	H	CH_3	—	—	6.89	6.57	OH	MeOH-d_4
16	—	—	H	CH_3	6.90	7.54	OH	MeOH-d_4
4[a]	Ac	Ac	—	—	7.11	6.93	AcO	$CDCl_3$
8	—	—	Ac	Ac	7.26	7.87	AcO	$CDCl_3$
1[a]	OH	OH	—	—	6.64	6.40	OH	DMSO-d_6
7	—	—	OH	OH	6.80	7.32	OH	DMSO-d_6

a) Prepared by the method as described.[7]

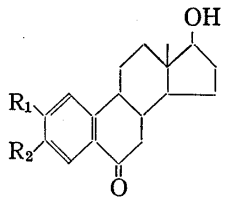
Two isomeric monomethyl ethers of the catechol (**7**) were prepared by the similar procedure. 2-Methoxyestradiol diacetate (**10**)[7] was converted to the corresponding 6-ketone, 2-methoxy-3,17β-dihydroxyestra-1,3,5(10)-trien-6-one diacetate (**11**), in the high yield of 51%. The yield of the isomer, 3-methoxy-2,17β-dihydroxy-estra-1,3,5(10)-trien-6-one diacetate (**15**) from 3-methoxy-2,17β-dihydroxyestra-1,3,5(10)-trien-6-one diacetate (**14**), on the other hand, was only 5%. The high yield of the ketones, **5** and **11**, is attributable to the activating effect of the electron-donating methoxy group on the *p*-benzylic position of the steroid nucleus. Similar result was obtained in the oxidation of 2-methoxy-3-deoxyestradiol derivative.[9] The mechanism of the oxidation reaction has been discussed already by Cambie *et al.*[10]

Saponification of **11** and **15** gave quantitatively the desired monomethyl ethers, 2-methoxy-3,17β-dihydroxyestra-1,3,5(10)-trien-6-one (**12**) and 3-methoxy-2,17β-dihydroxyestra-1,3,5(10)-trien-6-one (**16**), respectively.

The structure of these 6-ketones were confirmed by instrumental analyses. In nuclear magnetic resonance (NMR) spectra, the aromatic proton at C-4 of 6-ox-ocatechols is shifted about 1 ppm to down field compared with their parent catechols,[11] the results of which are shown in Table I.

In their ultraviolet (UV) spectra, these 6-oxo-catechol estrogens have three absorption maxima, λ_1, λ_2, and λ_3, as shown in Table II. For comparison, the results of other similar steroids, 17β-hydroxyestra-1,3,5(10)-trien-6-one(**17**), 2,17β-dihydroxyestra-1,3,5(10)-trien-6-one (**18**), 2-methoxy-17β-hydroxyestra-1,3,5(10)-trien-6-one (**19**), 6-oxo-estradiol (**20**), and 6-oxo-estradiol-3-methyl ether (**21**), are also given in Table II.

Table II. Ultraviolet Absorption Maximum (nm) and Molar Absorptivity (ε) of 17β-Hydroxyestra-1,3,5(10)-trien-6-one (**17**) and Its Derivatives[a]

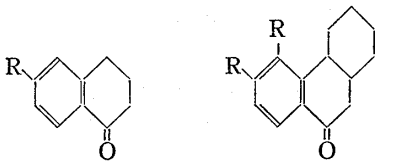


Compounds	R_1	R_2	λ_1	λ_2 Observed	λ_2 Calculated	λ_3
17[b]	H	H	—	251(11710)	—	294(2030)
18[c]	OH	H	231(11800)	288(13040)	276	—
19[c]	OCH_3	H	226(13900)	278(15200)	276	—
20[d]	H	OH	222(19700)	255(8460)	258	327(2900)
21[d]	H	OCH_3	224(15700)	257(5500)	258	326(1950)
6	OCH_3	OCH_3	234(18770)	276(10000)	283	316(7000)
7	OH	OH	236(14650)	281(9300)	283	322(6930)
12	OCH_3	OH	235(15710)	277(8630)	283	320(5540)
16	OH	OCH_3	235(17210)	281(9920)	283	317(8170)

a) Measured in EtOH except compound **21** (EtOH–$CHCl_3$).
b) Generous gift from Dr. Nambara, Tohoku Univ., Sendai.
c) I. Yoshizawa *et al.*, in reference 9.
d) R.C. Cambie *et al.*, in reference 10*b*.

9) I. Yoshizawa, K. Watanabe, K. Haga, and M. Kimura, *Yakugaku Zasshi*, **94**, 1484 (1974).
10) *a*) R.C. Cambie and T.D.R. Manning, *J. Chem. Soc.* (*C*), **1968**, 2603; *b*) R.C. Cambie, V.F. Carlisle, C.J. LeQuesne, and T.D.R. Manning, *ibid.*, **1969**, 1234; *c*) R.C. Cambie, V.F. Carlisle, and T.D.R. Manning, *ibid.*, **1969**, 1240; *d*) R.C. Cambie and V.F. Carlisle, *ibid.*, **1970**, 1706.
11) J. Fishman and J.S. Liang, *Tetrahedron*, **24**, 2199 (1968); T. Nambara, S. Honma, and S. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 474 (1970).

In these absorption spectra, the bands having maxima λ_1 and λ_3 may be attributable to those originated in the local excitation of π-electrones on aromatic ring, which are known as so-called E- and B-band, respectively. The remaining band having λ_2 corresponds to that due to the conjugated system (K-band), and where bathochtromic shift by the substituent(s) at the aromatic ring is observed. The calculation method of λ_{max} of these benzocyclanone derivatives has been established in such compounds as tetralone (**22**) and 10-oxo-octahydrophenanthrene (**24**).[12] Substitution at *p*-position against carbonyl group of these compounds by hydroxy or methoxy groups causes a bathochromic shift in the wave length 25 nm. The increment by the same substituents at *o*- and/or *m*-position is 7 nm. Thus, as these parent chromophores have absorption maxima at 249 nm, the λ_{max} of 6-methoxytetralone (**23**) is calculated as 249+25=274 nm (observed: 276 nm), and that of 3,4-dimethoxy-10-oxo-octahydrophenanthrene (**25**) as 249+25+7=281 nm (observed: 278 nm).



22: R=H **24**: R=H
23: R=OCH_3 **25**: R=OCH_3

Chart 2

Provided that application of the same additivity principle is possible for 6-oxo-estrogen derivatives, it may be reasonable to use the maximum value 251 nm of **17** as parent chromophore. Then their λ_{max} are easily obtained, which are shown in Tabel II. The calculated values are almost agreeable to the observed ones except **18**.

Other instrument analyses including mass (MS) spectra as well as infra-red (IR) spectra and also elemental analyses of these materials synthesized have supported their structures.

The utilization of the catechol (**7**) for biological studies is expected, especially in the behavior of *in vitro* and/or *in vivo* methylation. Other methyl ethers, 2,3-dimethoxy-17β-hydroxyestra-1,3,5(10)-trien-6-one (**6**), **12**, and **16**, may be useful as the authentic specimens for promoting these studies.

Experimental

Melting points were determined on a micro-hot stage (Mitamura) and are uncorrected. NMR spectra were recorded on JNM-PMX-60 spectrometer (JEOL) by using 5% solution containing tetramethylsilane as an internal standard, and s: singlet, m: multiplet, respectively. IR spectra were taken with JASCO-IR-A-2 (Nihon Bunko). MS spectra were taken with JMS-D 300 (JEOL) by direct insertion method. UV Spectra were measured with 200-20 spectrophotometer (Hitachi) with X-Y typed recorder. For column chromatography, SiO_2 (Merck, 70—230 mesh) was used.

2,3-Dimethoxy-17β-hydroxyestra-1,3,5(10)-trien-6-one Acetate (5)——To a solution of **3** derived from 2,3-dimethoxy-17β-hydroxyestra-1,3,5(10)-triene (**2**)[13] (569 mg) in acetic acid (8 ml) was added 15.1 ml of chromium trioxide reagent (9.0 g of CrO_3 in 60 ml of 90% acetic acid), and the mixture was stirred for 20 hr at room temperature. Excess reagent was decomposed by adding MeOH to the reaction mixture, which was then neutralized with 1 N KOH and extracted with ether. The combined extract was washed with water and dried over anhydrous Na_2SO_4. Removal of ether gave an oil (633 mg), which was chromatographed on SiO_2 column (2×30 cm). Elution with 70% $CHCl_3$ in benzene gave a crude material (157 mg), which was recrystallized from ether, mp 185—186°. *Anal.* Calcd. for $C_{22}H_{28}O_5$ (372.44): C, 70.94; H, 7.58. Found: C, 70.86; H, 7.47. NMR ($CDCl_3$) δ: 7.56 (1H, s, C_4-H), 6.88 (1H, s, C_1-H), 4.8—4.6 (1H, m, 17α-H), 3.96 (6H, s, 2×CH_3O), 2.02 (3H, s, CH_3COO), 0.84 (3H, s, 18-CH_3). IR ν_{max}^{KBr} cm^{-1}: 3050—2850 (CH), 1730 (AcO), 1670 (conjugated ketone), 1600 (aromatic). UV λ_{max}^{EtOH} nm (ε): 233 (18670), 277 (9960), 317 (7060). MS *m/e*: 372 (M^+).

2,3-Dimethoxy-17β-hydroxyestra-1,3,5(10)-trien-6-one (6)——A solution of **5** (72 mg) in 30 ml of methanolic (95%) 1 N KOH was allowed to stand overnight at room temperature. The mixture was then neutralized with 1 N HCl and extracted with ether. Fifty mg of the crude material was obtained, which was recrystallized from acetone to give colorless fine needles, mp 220—221°. *Anal.* Calcd. for $C_{20}H_{26}O_4$ (330.41): C, 72.70; H, 7.93. Found: C, 72.72; H, 7.90 NMR ($CDCl_3$) δ: 7.53 (1H s, C_4-1), 6.86 (1H, s, C_1-H), 4.3—4.1 (1H,

12) A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Chapter 3, Pergamon Press, Oxford, 1964, and references cited theirin.
13) J. Fishman, M. Tomasz, and R. Lehman, *J. Org. Chem.*, **25**, 585 (1960).

m, 17α-H), 3.90 (6H, s, 2×CH_3), 0.80 (3H, s, 18-CH_3). IR ν_{max}^{KBr} cm^{-1}: 3130 (OH), 3000—2850 (CH), 1725 (C=O), 1662 (conjugated ketone), 1600 (aromatic). UV λ_{max}^{EtOH} nm (ε): 234 (18770), 276 (10000), 316 (7000). MS *m/e*: 330 (M^+).

2,3-17β-Trihydroxyestra-1,3,5(10)-trien-6-one (7)——Compound **6** (88 mg) was treated with pyridine–HCl (1.3 g) at 200° for 15 min. After cooling, was added water to the reaction mixture, which was extracted with $CHCl_3$–MeOH (3:1). The combined extract was washed with water, dried over anhydrous Na_2SO_4. Removal of the solvent gave an oily material (163 mg), which was then submitted to column (1×20 cm) chromatography. Elution with 50% EtOAc in benzene gave 59 mg of crude material, which was recrystallized from MeOH–EtOAc to give fine needles, mp 273—275°. *Anal.* Calcd. for $C_{18}H_{22}O_4$ (302.36): C, 71.50; H, 7.33. Found: C, 71.34; H, 7.20. NMR (DMSO-d_6) δ: 7.32 (1H, s, C_4-H), 6.80 (1H, s, C_1-H), 0.66 (3H, s, 18-CH_3). IR ν_{max}^{KBr} cm^{-1}: 3600—3100 (OH), 3100—2800 (CH), 1662 (conjugated ketone), 1610 (aromatic). UV λ_{max}^{EtOH} nm (ε): 236 (14650), 281 (9300), 322 (6930). MS *m/e*: 302 (M^+).

2,3,17β-Trihydroxyestra-1,3,5(10)-trien-6-one Triacetate (8)——By the usual method, from 20 mg of **7** was obtained 23 mg of triacetate (**8**), mp 197—198.5° (MeOH). *Anal.* Calcd. for $C_{24}H_{28}O_7$ (428.46): C, 67.27; H, 6.59. Found: C, 67.40; H, 6.64. IR ν_{max}^{KBr} cm^{-1}: 2875 (CH), 1770, 1740 (AcO), 1680 (conjugated ketone). NMR ($CDCl_3$) δ: 7.87 (1H, s, C_4-H), 7.26 (1H, s, C_1-H), 4.8—4.6 (1H, m, 17α-H), 2.30—2.29 (6H, s, CH_3COO at C_2 and C_3), 2.06 (3H, s, 17β-CH_3COO), 0.82 (3H, s, 18-CH_3).

2-Methoxy-3,17β-dihydroxyestra-1,3,5(10)-trien-6-one Diacetate (11)——To a solution of 2-methoxyestra-1,3,5(10)-triene-3,17β-diol diacetate (**10**)[14] (6.3 g) in acetic aicd (150 ml) was added a chromium trioxide reagent (32 ml), and the mixture was stirred overnight at room temperature. By the same treatment as described above, the product was obtained as a white powder (6.15 g), which was submitted to column (3×40 cm) chromatography. From the eluate of 5% to 20% $CHCl_3$ in benzene, 1.32 g of crystalline material was obtained. Recrystallization from MeOH gave fine needles, mp 215—217°. *Anal.* Calcd. for $C_{23}H_{28}O_6$ (400.45): C, 69.98; H, 7.05. Found: C, 70.03; H, 7.11. NMR ($CDCl_3$) δ: 7.73 (1H, s, C_4-H), 6.93 (1H, s, C_1-H), 4.9—4.6 (1H, m, 17α-H), 3.90 (3H, s, CH_3O), 2.30 (3H, s, CH_3COO), 2.06 (3H, s, 17β-CH_3COO), 0.83 (3H, s, 18-CH_3). IR ν_{max}^{KBr} cm^{-1}: 3000—2850 (CH), 1770, 1730 (CH_3COO), 1670 (conjugated ketone), 1603 (aromatic). UV λ_{max}^{EtOH} nm (ε): 226.5 (24030), 273 (15880), 299 (shoulder, 8820). From the eluates of 20% $CHCl_3$ in benzene, a crude material was obtained (1.95 g), which was shown to be a mixture of mono- and diacetate (C_3-acetyl group is removed) by NMR. So, the mixture was treated with pyridine–Ac_2O to give 1.98 g of **11**. Therefore, the yield of the ketone (**11**) was 3.3 g (51%).

2-Methoxy-3,17β-dihydroxyestra-1,3,5(10)-trien-6-one (12)——Saponification of **11** (400 mg) was carried out by the same procedure as described above, and 301 mg of crude product was obtained. Recrystallization by $CHCl_3$ gave colorless fine needles, mp 226—227°. *Anal.* Calcd. for $C_{19}H_{24}O_4$ (316.38): C, 72.12; H, 7.65. Found: C, 72.27; H, 7.35. IR ν_{max}^{KBr} cm^{-1}: 3550—3100 (OH), 3000—2850 (CH), 1660 (conjugated ketone), 1610 (aromatic). MS *m/e*: 316 (M^+), 298 (M^+-H_2O). NMR (MeOH-d_4) δ: 7.40 (1H, s, C_4-H), 6.95 (1H, s, C_1-H), 3.96 (3H, s, CH_3O), 0.80 (3H, s, 18-CH_3). UV λ_{max}^{EtOH} nm (ε): 235 (15710), 277 (8630), 320 (5540).

3-Methoxy-2,17β-dihydroxyestra-1,3,5(10)-trien-6-one Diacetate (14)——From 1.07 g of 2-hydroxyestradiol-3-methyl ether (**13**)[9] was obtained **14** (1.20 g) by the usual method, mp 135—136.5° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3000—2800 (CH), 1760, 1727 (Ac), 1610 (aromatic).

3-Methoxy-2,17β-dihydroxyestra-1,3,5(10)-trien-6-one Diacetate (15)——By the same procedure as described in the oxidation reaction, from 840 mg of **14** was obtained a product as an oily material (418 mg), which was chromatographed on 2×20 cm column. Elution with benzene to 7% $CHCl_3$ in benzene gave a crude material (137 mg), which was then submitted to preparative thin-layer chromatography (Wakogel B-5F, thickness: 0.75 mm) using developing solvent: cyclohexane–acetone (3:1), developing three times. The ketone (**15**) was obtained as a fine needles, mp 154.5—156° (MeOH). *Anal.* Calcd. for $C_{23}H_{28}O_6$ (400.45): C, 69.98; H, 7.05. Found: C, 69.94; H, 7.10. IR ν_{max}^{KBr} cm^{-1}: 3000—2850 (CH), 1770, 1730 (AcO), 1680 (conjugated ketone), 1610 (aromatic). NMR ($CDCl_3$) δ: 7.66 (1H, s, C_4-H), 7.10 (1H, s, C_1-H), 4.8—4.6 (1H, m, 17α-H), 3.86 (3H, s, CH_3O), 2.33 (3H, s, CH_3COO at C_2), 2.06 (3H, s, 17β-CH_3COO), 0.83 (3H, s, 18-CH_3). UV λ_{max}^{EtOH} nm (ε): 223.5 (22330), 259 (9730), 315 (4000).

3-Methoxy-2,17β-dihydroxyestra-1,3,5(10)-trien-6-one (16)——Saponification of **15** (38.2 mg) by the same procedure as described above gave material, 30 mg. Crystallization by a mixture of acetone and cyclohexane afforded fine needles, mp 247—249°. *Anal.* Calcd. for $C_{19}H_{24}O_4$: C, 72.12; H, 7.65. Found: C, 72.10; H, 7.63. IR ν_{max}^{KBr} cm^{-1}: 3550, 3250—3000 (OH), 2900—2800 (CH), 1650 (conjugated ketone), 1600 (aromatic). NMR (MeOH-d_4) δ: 7.54 (1H, s, C_4-1), 6.90 (1H, s, C_1-H), 3.90 (3H, s, CH_3O), 0.80 (3H, s, 18-CH_3). UV λ_{max}^{EtOH} nm (ε): 235 (17210), 281 (9920), 317 (8170). MS *m/e*: 316 (M^+), 298 (M^+-H_2O).

Acknowledgement The authors wish to thank Mr. H. Watanabe and Miss F. Iwamoto for technical assistance. They wish to thank the staffs of the central analytical laboratory of Faculty of Pharmaceutical Sciences, Hokkaido University, for elemental analyses.

14) J. Fishman, *J. Am. Chem. Soc.*, **80**, 1213 (1958).