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Isolation and Characterization of Urinary Metabolites of 16α-Chloroestrone Methyl Ether in the Rabbit[1,2]

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The metabolic fate of 16α-chloroestrone methyl ether (I), which is used as a lipid-shifting drug, has been investigated in the rabbit. Five principal metabolites were separated from the urine specimen collected after oral administration of I. These metabolites were identified as 16α-chloro-17α-estradiol (II), estrone (III), 16β-chloro-17α-estradiol (IV), 17α-estradiol (V) and 16,17-epiestriol (VI) by direct comparison with the authentic samples, respectively (see Chart 1). The biochemical significance of *in vivo* transformation hereby observed has been discussed. The synthesis of the reference compounds for comparison with the metabolites has also been described.

16α-Chloroestrone methyl ether (I) is now widely used for the clinical states associated with hypercholesterolemia as a lipid-shifting drug.[4] As a part of our studies on the modified steroids[5] we attempted to explore the metabolic fate of this drug. The present paper deals with the isolation and characterization of the urinary metabolites of I administered to the rabbit.

In a typical run a suspension of the steroid in Tween 80 was orally given to an adult male rabbit. The urine specimen collected for the following 48 hours was adsorbed on Amberite XAD-2 resin and then washed with water for removal of the nonsteroidal substances. The crude metabolites eluted with methanol were divided into the unconjugated steroid, glucuronide and sulfate fractions in the usual manner. The result of thin-layer chromatography (TLC) indicated that the enzymatic hydrolyzate of glucuronide fraction consisted of several metabolites (see Fig. 1)[6] whereas any metabolite could not be detected in the unconjugated and sulfate fractions. Separation of each metabolite was efficiently achieved by the preparative TLC upon multiple development. All the metabolites showed a positive Folin-Ciocalteu test indicating the presence of a free hydroxyl group on ring A.

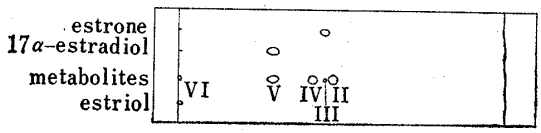

Fig. 1. Thin-Layer Chromatogram of the Main Metabolites of 16α-Chloroestrone Methyl Ether in Rabbit Urine

plate: Silica gel G (E. Merck AG)
solvent system: benzene–ether (5:1)
staining: conc. H_2SO_4, Folin–Ciocalteu reagent

1) This paper constitutes Part XLVIII of the series entitled "Analytical Chemical Studies on Steroids"; Part XLVII: T. Nambara, S. Akiyama, and S. Honma, *Chem. Pharm. Bull.* (Tokyo), **19**, 1727 (1971).
2) In this paper the following trivial names are used: 3-deoxyestrone=estra-1,3,5(10)-trien-17-one; estrone=3-hydroxyestra-1,3,5(10)-trien-17-one; estradiol=estra-1,3,5(10)-triene-3,17β-diol; 17α-estradiol=estra-1,3,5(10)-triene-3,17α-diol; estriol=estra-1,3,5(10)-triene-3,16α,17β-triol; 16-epiestriol=estra-1,3,5(10)-triene-3,16β,17β-triol; 17-epiestriol=estra-1,3,5(10)-triene-3,16α,17α-triol; 16,17-epiestriol=estra-1,3,5(10)-triene-3,16β,17α-triol.
3) Location: *Aobayama, Sendai.*
4) G.P. Mueller, W.F. Johns, D.L. Cook, and R.A. Edgren, *J. Am. Chem. Soc.*, **80**, 1769 (1958).
5) *a*) T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1200 (1970); *b*) T. Nambara and Y.H. Bae, *ibid.*, **18**, 2119 (1970); *c*) T. Nambara and H. Takahashi, *ibid.*, **18**, 2309 (1970); *d*) T. Nambara and M. Numazawa, *ibid.*, **19**, 990 (1971).
6) These metabolites are numerically designated as II to VI accoring to the order of increasing polarity.

First, metabolite II was isolated as colorless needles. On the mass spectrum a molecular ion peak appeared at *m/e* 306 accompanied with an isotope peak (*m/e* 308) suggesting the retention of chlorine at C-16 and *in vivo* reduction of the oxo function to the hydroxyl group. Treatment with methanolic sodium hydroxide yielded a ketosteroid which was identified as estrone. These results implied that the metabolite would be the 16,17-*cis*-chlorohydrin. Since the four isomeric 16,17-chlorohydrins were not documented in the literatures, these compounds were synthesized by the usual method. As was shown in Table I four possible isomers could obviously be differentiated one another by TLC and gas–liquid chromatography (GLC). In actuality metabolite II proved to be identical with 16α-chloro-17α-estradiol in every respect by comparison with the authentic sample.

Table I. Chromatographic Data of Isomeric 16-Chloroestratriene-3,17-diols

Compound	GLC[a] (TFA)	TLC[b] (Free)
16α-Chloroestradiol	0.42	0.37
16β-Chloroestradiol	0.55	0.44
16α-Chloro-17α-estradiol	0.49	0.56
16β-Chloro-17α-estradiol	0.37	0.40

a) Retention time relative to cholestane (90.0 min) was given.
column: 5% SE-52 on Chromosorb W (60—80 mesh)(6.375 m)
temperature: column 230°, injection chamber 250°, detector 250°
carrier gas: N_2 flow rate 40 ml/min
b) *Rf* value was given.
developing solvent: benzene–AcOEt (6:1)

In the mother liquor of metabolite II the presence of the second metabolite, III, was suggested. Unfortunately this substance could not be separated in the crystalline state because of very small amount available. On the basis of the chromatographic behaviors and color reactions with conc. sulfuric acid and Zimmermann reagent was drawn a conclusion that metabolite III should be estrone.

Then metabolite IV was obtained as colorless needles. The mass spectrum of this compound was quite similar to that of II indicating that the metabolite would be isomeric. On treatment with methanolic alkali metabolite IV was led to the 16,17-epoxide which was

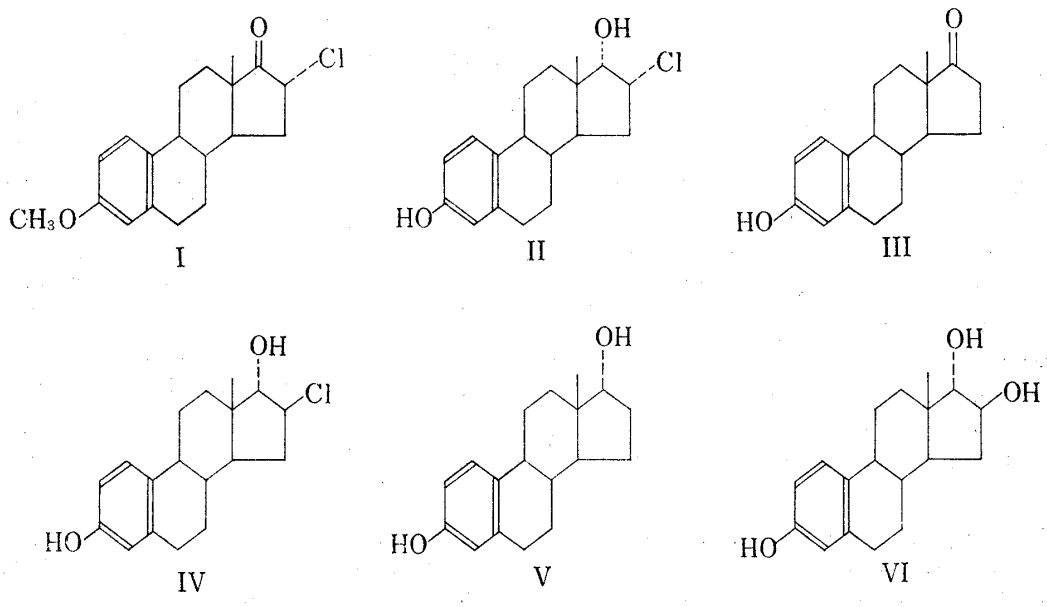

Chart 1

characterized by TLC. These evidences together prompted us to compare with two possible 16,17-*trans*-chlorohydrins. Thus the assignment of the structure 16β-chloro-17α-estradiol to metabolite IV was definitely established by usual criteria.

Recrystallization of the eluate which exhibited the polarity of the diol gave metabolite V as colorless needles. This substance was evidently distinguishable from estradiol and seemed very likely to be the 17α-epimer. In fact metabolite V proved to be entirely identical with the authentic 17α-estradiol in all respects, *i.e.* thin-layer and gas chromatographic constants, mixed melting point, and infrared (IR) spectra.

Of the principal metabolites the most polar one, metabolite VI, was separated as an oily substance. The chromatographic behaviors indicated the existence of two additional hydroxyl functions besides a phenolic group. Considering the results on the metabolic fate of 3-deoxyestrone,[5a] this substance was assumed to be a 3,16,17-triol. Hence examinations were made on the chromatographic behaviors of four isomeric 3,16,17-triols. In consequence these isomers could be separated with success by TLC and GLC as indicated in Table II. According to the procedure thus established the identity of the metabolite VI and authentic 16,17-epiestriol was unequivocally justified.

Table II. Chromatographic Data of Isomeric Estratriene-3,16,17-triols

Compound	GLC[a] (TFA)	TLC[b] (Free)	TLC[b] (Acetate)
Estriol	0.23	0.28	0.42
16-Epiestriol	0.36	0.62	0.54
17-Epiestriol	0.48	0.71	0.72
16,17-Epiestriol	0.18	0.39	0.47

a) Retention time relative to cholestane (45.2 min) was given.
column: 1.5% OV-17 on Shimalite W (60—80 mesh) (1.875 m)
temperature: column 200°, injection chamber 250°, detector 250°
carrrrier gas: N_2 flow rate 60 ml/min
b) *Rf* value was given.
developing solvent: ether–AcOEt (9:1) (Free), benzene–ether (5:1)(Acetate)

Discussion

First it is to be noted that reductive elimination of chlorine at C-16 did take place resulting in the formation of 17α-estradiol, 16,17-epiestriol and estrone. The quantitation of the urinary metabolites by gas chromatography, however, demonstrated that 16α-chloro-17α-estradiol formed 70% of the excreted amount.[7] This finding led to the assumption that an introduction of a halogen into C-16 position of the estrogen may serve to prevent the normal biotransformation. The enzymatic dehalogenation has already been demonstrated with Bromural ((2-bromo-3-methylbutyryl)urea) and α-bromoacylureide having an active halogen.[8] On the other hand the nonenzymatic dehalogenation has also been reported with some halogeno compounds.[9] It is therefore an attractive problem to clarify whether or not the enzymatic mechanism is operative for dehalogenation in the case of I.

Demethylation of C-3 methoxy group in the A-ring aromatic steroid proceeded readily in the rabbit. This result is fairly consistent with the previous finding on the analogous modified steroids such as Mestranol (17α-ethynylestradiol 3-methyl ether).[10] In a separate experiment

7) T. Nambara, Y.H. Bae, and M. Nokubo, *J. Chromatog.*, **60**, 418 (1971).
8) T.C. Butler, *J. Pharmacol. Exptl. Therap.*, **143**, 23 (1964); T. Narafu, *Yakugaku Zasshi*, **87**, 357 (1967).
9) H.G. Bray, W.V. Thorpe, and D.K. Vallance, *Biochem. J.*, **51**, 193 (1952).
10) M.T. Abdel-Azid and K.I.H. Williams, *Steroids.*, **13**, 809 (1969).

with estrone methyl ether the similar result was also obtained in the rabbit.[11] In addition demethylation of C-3 methoxy group appears to proceed with more ease than that of C-2.[12]

The occurrence of 16β-chloro-17α-estradiol is of particular interest in suggesting that configuration of C_{16}-chlorine was changed from α to β. To the best of our knowledge this appears to be the first demonstration of *in vivo* epimerization of halogen. It is sufficiently substantiated that the steroidal 16α-halo-17-ketone is readily epimerized to yield an equilibrium mixture.[13] On the other hand the existence of the epimerase in the animal kingdom has also been reported.[14] At present the mechanism of this novel biotransformation still remains unknown. It is hoped that further work in progress in this laboratory will provide the more precise knowledge on the mode of *in vivo* epimerization.

With regard to the conjugate form it is noteworthy that all the principal metabolites were found in the glucuronide fraction. Recently, Layne and his co-workers disclosed the occurrence of a double conjugate of 17α-estradiol, that is 17-N-acetylglucosaminide-3-glucuronide, in rabbit urine after a large dosage of estrone benzoate.[15] In the present case the excretion of any other conjugate than the glucuronide could not be detected. This result is in accord with the previous finding on the metabolism of 3-deoxyestrone in the rabbit.[5d]

Experimental[16]

Animal——An adult male rabbit weighing about 2 kg was housed in a cage that was designed to minimize fecal contamination of the urine.

Administration of 16α-Chloroestrone Methyl Ether (I) and Collection of Urine——A single dose of a suspension of I (200 mg) in Tween 80 was injected into a stomach through a catheter, and the 48 hr urine specimen was collected in a bottle containing a few drops of toluene as a preservative.

Separation of Metabolites——The pooled urine was passed through a column of Amberlite XAD-2 resin (20×2 cm), washed with H_2O (3 liter) and then eluted with MeOH (500 ml). The eluate thus obtained was dissolved in H_2O and extracted with ether for the separation of the free steroids. The aqueous phase was adjusted to pH 5 with 50% H_2SO_4, then to pH 4.7 with 0.1M acetate buffer (10 ml/100 ml of urine) and incubated with beef-liver β-glucuronidase (Tokyo Zōkikagaku, Co.) (500 Fishman U/ml) at 37° for 5 days. The hydrolyzate was extracted with ether three times and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave the glucuronide fraction. The remaining aqueous layer was then brought to pH 1 with 50% H_2SO_4, saturated with NaCl (20 g/100 ml) and extracted with AcOEt. The organic phases were combined and allowed to stand at 37° for 24 hr. The extract was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the sulfate fraction was obtained. Each metabolite was separated by the preparative TLC using silica gel HF (E. Merck AG) as an adsorbent and eluted with AcOEt.

Thin-layer Chromatography——TLC was carried out on silica gel G (E. Merck AG) plate by the following systems: TL-I=benzene–ether (5:1); TL-II=benzene–AcOEt (6:1); TL-III=ether–AcOEt (9:1), and *Rf* value was given.

Gas Liquid Chromatography——The apparatus used for this work was a Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector and a U-shaped stainless steel tube (3 mm *i.d.*) packed with 3% SE-30 on Chromosorb W (60—80 mesh) (A), 1.5% OV-17 on Shimalite W (60—80 mesh) (B), 2% OV-17 on Shimalite W (60—80 mesh) (C) or 5% SE-52 on Chromosorb W (60—80 mesh) (D). The temperatures of column, detector and injection chamber were kept at 230°, 250° and 250° (condition GC-I) or 200°, 250° and 250° (condition GC-II), respectively. N_2 was used as a carrier gas at a flow rate of 40 ml/min unless otherwise specified. Trifluoroacetyl (TFA)and trrimethylsilyl (TMS) derivatives

11) T. Nambara and M. Nokubo, to be published.
12) T. Nambara, S. Honma, and K. Kanayama, to be published.
13) J. Fajkoš, *Collection Czech. Chem. Commun.*, **20**, 312 (1955); *idem*, *J. Chem. Soc.*, **1959**, 3966.
14) R.D.H. Heard, *Recent Progr. Hormone Res.*, **4**, 25 (1949); H. Breuer and G. Pangels, *Acta Endocrinol.*, **33**, 532 (1960).
15) D.C. Collins, H. Jirku, and D.S. Layne, *J. Biol. Chem.*, **243**, 2928 (1968), and references quoted therein.
16) Melting points were taken on a micro hot-stage apparatus and are uncorrected. IR spectral measurements were run on JASCO Model IR-S spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained by Hitachi Model R-20 spectrometer at 60 Mc: the chemical shifts are quoted as ppm downfield from tetramethylsilane used as an internal standard. Mass (MS) spectra were measured by Hitachi Model RMU-7 spectrometer.

were prepared according to the procedures of VandenHeuvel, *et al.*[17] and Sweeley, *et al.*,[18] respectively. Retention time relative to cholestane, t_R, was given.

Synthesis of Reference Compounds

16β-Chloroestradiol——To a solution of 16α-chloroestrone acetate (200 mg) in MeOH (25 ml) was added 10% Na_2CO_3 (5 ml) and stirred at room temperature for 1 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave an oily residue. To a solution of this crude product in THF (20 ml) was added $LiAlH_4$ (30 mg) under ice-cooling and allowed to stand for 30 min. The reaction mixture was decomposed with moist ether and acidified with 10% HCl. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up an oily product obtained was submitted to the preparative TLC using benzene–AcOEt (6:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.44) and recrystallization of the eluate from MeOH gave 16β-chloroestradiol (20 mg) as colorless plates. mp 183—184°. $[\alpha]_D^{21}$ +64.5° (c=0.15, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{23}O_2Cl \cdot 1/4 H_2O$: C, 69.47; H, 7.61. Found: C, 69.42, 69.44; H, 7.78, 7.83. IR ν_{max}^{KBr} cm^{-1}: 3100—3500 (OH). Treatment with Raney Ni gave estradiol, which was characterized by TLC. When refluxed with methanolic NaOH, elimination of hydrogen chloride proceeded readily resulting in the formation of estrone.

16β-Chloro-17α-estradiol (IV)——To a solution of 3-benzyloxy-16α,17α-epoxyestra-1,3,5(10)-triene (50 mg) in dioxane (10 ml) was added conc. HCl (0.05 ml) and allowed to stand at room temperature for 30 min. The resulting solution was diluted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up an oily residue obtained was submitted to the preparative TLC using benzene–ether (5:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.90) was eluted, and the eluate was subjected to further step without purification. A solution of this crude product (30 mg) in AcOEt (20 ml) was shaken with 10% Pd/C (10 mg) under a stream of H_2 gas for 2 hr. After removal of the catalyst by filtration the filtrate was concentrated *in vacuo* to give a crystalline product. Recrystallization from benzene gave IV (20 mg) as colorless needles. mp 173—174°. $[\alpha]_D^{21}$ +71.4° (c=0.10, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{23}O_2Cl \cdot {}^2/_3C_6H_6$: C, 73.62; H, 7.58. Found: C, 73.46, 73.51, 73.89; H, 7.68, 7.66, 7.71. Mass Spectrum m/e: 306 (M^+), 278 (M-28), 220.

16α-Chloro-17α-estradiol (II), 16α-Chloroestradiol——To a solution of 16α-chloroestrone acetate (100 mg) in THF (6 ml) was added dropwise a solution of $LiAlH_4$ (30 mg) in THF (8 ml) under ice-cooling and allowed to stand for 30 min. The reaction mixture was decomposed with moist ether and acidified with 10% HCl. The organic layer was separated, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up an oily product obtained was submitted to the preparative TLC using benzene–ether (5:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.53) and recrystallization of the eluate from aq. MeOH gave II (26 mg) as colorless needles. mp 228—229°. $[\alpha]_D^{21}$ +66.9° (c=0.10, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{23}O_2Cl$: C, 70.46; H, 7.56. Found: C, 70.32; H, 7.65. IR ν_{max}^{KBr} cm^{-1}: 3200—3600 (OH). Mass Spectrum m/e: 306 (M^+), 278 (M-28), 220. Treatment with Raney Ni gave V, which was characterized by TLC. When refluxed with methanolic NaOH, elimination of hydrogen chloride proceeded readily resulting in the formation of III. Elution of the adsorbent corresponding to the spot (*Rf* 0.31) and recrystallization of the eluate from MeOH gave 16α-chloroestradiol (50 mg) as colorless prisms. mp 213—214°. $[\alpha]_D^{21}$ +90.5° (c=0.11, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{23}O_2Cl$: C, 70.46; H, 7.56. Found: C, 70.77; H, 7.60. IR ν_{max}^{KBr} cm^{-1}: 3200—3600 (OH). Mass Spectrum m/e: 306 (M^+), 220. To a solution of 16α-chloroestradiol (150 mg) in MeOH (10 ml) was added 5% methanolic NaOH (4 ml) and refluxed for 24 hr. The resulting solution was diluted with CH_2Cl_2, washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up a crude product obtained was submitted to the preparative TLC using benzene–AcOEt (6:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.62) and recrystallization of the eluate from AcOEt gave 16β,17β-epoxyestra-1,3,5(10)-trien-3-ol (50 mg) as colorless prisms. mp 199—201°. $[\alpha]_D^{18}$ +100.0° (c=0.07, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 80.28; H, 8.28. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 3.27 (1H, d, J=3.7 cps, 17α-H), 3.55 (1H, q, J=2.7, 3.7 cps, 16α-H), 5.42 (1H, m, 3-OH). Fishman, *et al.* prepared this compound by the different route (reported: mp 200—204°, $[\alpha]_D^{25}$ +119°).[19]

3-Benzyloxy-16α,17α-epoxyestra-1,3,5(10)-triene——To a solution of 3-benzyloxyestra-1,3,5(10), 16-tetraene[20] (340 mg) in $CHCl_3$ (20 ml) was added *m*-chloroperbenzoic acid (250 mg) and allowed to stand at 4° for 12 hr. The resulting solution was washed with 0.1N $Na_2S_2O_3$, 5% $NaHCO_3$ and H_2O, successively and dried over anhydrous Na_2SO_4. After usual work-up a crystalline product obtained was recrystallized from MeOH to give 3-benzyloxy-16α,17α-epoxyestra-1,3,5(10)-triene (240 mg) as colorless needles. mp 112—113°. $[\alpha]_D^{16}$ +69.4° (c=0.11, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{28}O_2$: C, 83.29; H, 7.83. Found: C, 83.17; H, 7.96. NMR (4% solution in CCl_4) δ: 0.73 (3H, s, 18-CH_3), 2.95 (1H, d, J=3 cps, 17β-H), 3.18 (1H, d,

17) W.J.A. VandenHeuvel, J. Sjövall, and E.C. Horning, *Biochim. Biophys. Acta*, **48**, 596 (1961).
18) C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
19) J. Fishman and W.R. Biggerstaff, *J. Org. Chem.*, **23**, 1190 (1958).
20) T. Nambara, M. Numazawa, and H. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1725 (1969).

J=3 cps, 16β-H), 4.90 (2H, s, $C_6H_5CH_2$-).

Identification of Metabolites

16α-Chloro-17α-estradiol (Metabolite II)——Elution of the adsorbent corresponding to the spot (TL-II 0.56) with AcOEt and recrystallization of the eluate from aq. MeOH gave metabolite II (8 mg) as colorless needles. mp 228—229°. This substance gave a positive Folin–Ciocalteu test. Mass Spectrum m/e: 308 (M+2), 306 (M^+), 278 (M-28), 220 (M-86), 263 (M-93), 159. IR ν_{max}^{KBr} cm^{-1}: 3200—3600 (OH). Refluxing with 5% methanolic NaOH for 1 hr gave III, which was identified by TLC (TL-II 0.45). Mixed mp measurement on admixture with the authentic specimen and IR spectra comparison showed the identity of the two samples. GLC: GC-IA (TMS) 0.98, GC-IIA (TFA) 0.44, GC-ID (TFA) 0.49.

Estrone (Metabolite III)——Metabolite III was obtained from the mother liquor of metabolite II as a colorless oil. This substance gave positive results with both Folin–Ciocalteu and Zimmermann reagents. TLC: TL-I 0.38, TL-II 0.45. GLC: GC-IA (TMS) 0.66, GC-IIA (TFA) 0.28, GC-IC (TMS) 0.91, GC-IC (TFA) 0.50. Usual acetylation with pyridine and Ac_2O gave the acetate, which was identified by TLC (TL-II 0.58).

16β-Chloro-17α-estradiol (Metabolite IV)——Elution of the adsorbent corresponding to the spot (TL-II 0.40) with AcOEt and recrystallization of the eluate from $CHCl_3$ gave metabolite IV (1 mg) as colorless needles. mp 173—174°. This substance gave a positive Folin–Ciocalteu test. Mass Spectrum m/e: 308 (M+2), 306 (M^+), 278 (M-28), 220 (M-86), 213 (M-93), 159. Refluxing with 5% methanolic NaOH for 3 hr gave 16α, 17α-epoxyestra-1,3,5(10)-trien-3-ol, which was identified by TLC (TL-II 0.59). Mixed mp on admixture with the authentic sample showed no depression. GLC: GC-ID (TFA) 0.37.

17α-Estradiol (Metabolite V)——Elution of the adsorbent corresponding to the spot (TL-II 0.24) with AcOEt and recrystallization of the eluate from aq. MeOH gave metabolite V (5 mg) as colorless needles. mp 220—222°. This substance gave a positive Folin–Ciocalteu test. IR ν_{max}^{KBr} cm^{-1}: 3200—3600 (OH). Mixed mp measurement on admixture with the authentic specimen and IR spectra comparison showed the identity of the two samples. Usual acetylation with pyridine and Ac_2O gave the diacetate, which was identified by TLC (TL-II 0.79). GLC: GC-IA (TMS) 0.59, GC-IC (TMS) 0.62.

16,17-Epiestriol (Metabolite VI)——Elution of the adsorbent corresponding to the spot (TL-III 0.39) with AcOEt gave metabolite VI (trace) as a colorless oil. This substance gave a positive Folin–Ciocalteu test. Usual acetylation with pyridine and Ac_2O gave the triacetate, which was identified by TLC (TL-I 0.47). GLC: GC-IIB (N_2 60 ml/min) (TFA) 0.18.

Acknowledgement The authors are indebted to all the staffs of the central analytical laboratory of this Institute for elemental analyses, NMR and mass spectral measurements. This work was supported in part by a Grant-in-Aid from Iatrochemical Research Foundation, which is gratefully acknowledged.