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# On the Reactivity of Two Hydroxyl Groups of Catechol Estrogen[1]

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Of the two hydroxyl groups of catechol estrogen, C-2 phenolic functional is six times more reactive as the adjacent one at C-3 position toward the Smiles rearrangement of 2-benzoyl-4-nitrophenyl group. The reasons for this result are discussed.

The important estrogenic hormone estradiol is metabolized to 2-hydroxyestrone (I).[3] Although this catechol estrogen has two "indistinguishable" phenolic hydroxyl groups,[4] the main biological transformation is extremely specific for only one hydroxyl group of two adjacent phenolic functionals of ring-A. The followings are typical examples: catechol-O-methyltranslation of 2-hydroxyestrone in man occurs only at C-2 position to give 2-methoxyestrone (II) without any evidence for the formation of 3-methoxycompound (III),[5] and further, the usual biological conjugations of this catechol estrogen are also extremely stereoselective, namely, glucosiduronation in the animal such as rat as well as hamster[6] and guinea pig,[7] occurs exclusively at C-2 phenolic hydroxyl group, and sulfation of the same substrate in rat[8] and human being[9] also at the same position. These examples of enzymatic biotransformation should, of course, be derived from the results influenced by sterical environment between substrate and enzyme molecule. However, if there is a considerable difference in the acidity or reactivity between two phenolic functionals, a situation of considerable biological consequences described above may be dominated by character difference of the two hydroxyl groups as well as sterical relation. Typical examples are found in the important catecholamines in which O-methyltranslation takes place mainly at the hydroxyl group whose basicity is greater than the other one.[10] As was described by Fishman, *et al.*,[4] there was no distinct reactivity difference to be found in the two phenolic groups of this catechol estrogen on the synthesis of simple derivatives such as benzylation or acetylation as described below.

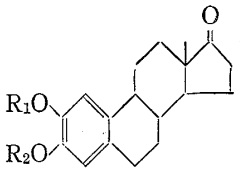
In this report, the authors describe that the significant difference in the chemical reactivity of two phenolic hydroxyl groups of this catechol estrogen appeared in the Smiles rearrangement of 2-benzoyl-4-nitro-phenyl ethers of catechol estrogen typed (XII) and or (XIV).

1) A part of this work was presented at the 91th Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo, Hokkaido.*
3) J. Fishman, *J. Clin. Endocr. Metab.*, **23**, 207 (1963); J. Fishman, H. Guzik, and L. Hellman, *Biochemistry*, **9**, 1593 (1970).
4) J. Fishman, M. Miyazaki, and I. Yoshizawa, *J. Am. Chem. Soc.*, **89**, 7147 (1967); M. Miyazaki, I. Yoshizawa, and J. Fishman, *Biochemistry*, **8**, 1669 (1969).
5) J. Fishman, R.I. Cox, and T.F. Gallagher, *Arch. Biochem. Biophys.*, **90**, 318 (1960).
6) K.I.H. Williams, *Steroids*, **15**, 105 (1970).
7) I. Yoshizawa, K. Fujimori, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **19**, 2431 (1971).
8) R. Knuppen and H. Breuer, *Adv. Biosciences*, **3**, 81 (1969).
9) I. Yoshizawa and J. Fishman, *J. Clin. Endocr. Metab.*, **29**, 1123 (1969).
10) J. Axelrod, "Transmethylation and Methionine Biosynthesis" ed. by S.K. Shapiro and F. Schlenk, Univ. Chicago Press, 1965, pp. 71—84.

## Result and Discussion

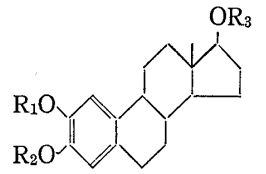
At first, the preparations of ordinary derivatives of catechol estrogen such as benzylation or acetylation were carried out. Benzylation of (I) with benzyl chloride equivalent to steroid and potassium carbonate in refluxing ethanol under nitrogen stream gave isomeric mono-benzyl ethers (IV) and (V) with a small amount of di-benzyl ether (VI). The ratio of (IV) to (V) was 1.4:1, with a slightly predominant amount of 2-substituted derivative. Acetylation of (I) by using equivalent acetic anhydride to steroid gave isomeric mono-acetates, (VII) and (VIII), with also a little predominance of 2-substituent. On the bases of these findings, the C-2 hydroxyl group is slightly more reactive than the C-3 hydroxyl, but it may be concluded that those two phenolic hydroxyl groups are indistinguishable functionally as suggested by Fishman, *et al.*[4]

In order to get a benzyl ether (XIII) from our sheer necessity, compound (XII) which is an important intermediate to 2-hydroxylated estrogen and its derivatives,[11] was refluxed in ethanol containing benzyl chloride and potassium carbonate[12] for 8 hours. In this experiment, the occurrence of the migration of 2-benzoyl-4-nitrophenyl group (abbreviated as BNP-group) was expected by the report of Loudon, *et al.*[13] in that BNP-group migrates easily from weaker basic hydroxyl group to stronger one in the presence of alkali by famous Smiles rearrangement.[14]



I: $R_1$=H, $R_2$=H
II: $R_1$=$CH_3$, $R_2$=H
III: $R_1$=H, $R_2$=$CH_3$
IV: $R_1$=$C_6H_5$-$CH_2$-, $R_2$=H
V: $R_1$=H, $R_2$=$C_6H_5$-$CH_2$-
VI: $R_1$=$C_6H_5$-$CH_2$-, $R_2$=$C_6H_5$-$CH_2$-
VII: $R_1$=$CH_3$-CO-, $R_2$=H
VIII: $R_1$=H, $R_2$=$CH_3$-CO-
IX: $R_1$=$CH_3$-CO-, $R_2$=$CH_3$
X: $R_1$=$CH_3$, $R_2$=$CH_3$-CO-

Chart 1



XI: $R_1$=H, $R_2$=H-, $R_3$=H
XII: $R_1$=H, $R_2$=BNP-, $R_3$=$CH_3$-CO-
XIII: $R_1$=$C_6H_5$-$CH_2$-, $R_2$=BNP-, $R_3$=H
XIV: $R_1$=BNP-, $R_2$=H, $R_3$=$CH_3$-CO-
XV: $R_1$=BNP-, $R_2$=$C_6H_5$-$CH_2$-, $R_3$=H
XVI: $R_1$=$C_6H_5$-$CH_2$-, $R_2$=H, $R_3$=H
XVII: $R_1$=H, $R_2$=$C_6H_5$-$CH_2$-, $R_3$=H
XVIII: $R_1$=$C_6H_5$-$CH_2$-, $R_2$=$C_6H_5$-$CH_2$-, $R_3$=H
XIX: $R_1$=$CH_3$, $R_2$=BNP-, $R_3$=H
XX: $R_1$=BNP-, $R_2$=$CH_3$, $R_3$=H
XXI: $R_1$=$CH_3$, $R_2$=H, $R_3$=H
XXII: $R_1$=H, $R_2$=$CH_3$, $R_3$=H

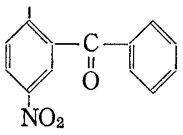
BNP-: 

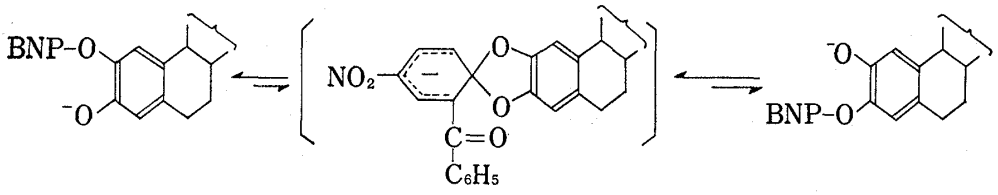
Chart 2

But in the present case, migration should be occurred in divergence to give a mixture of two kinds of benzyl ethers (XIII) and (XV) in the almost equol ratio because there was

11) J. Fishman, *J. Am. Chem. Soc.*, **80**, 1213 (1958); J. Fishman, M. Tomasz, and R. Lehman, *J. Org. Chem.*, **25**, 585 (1960).
12) Potassium carbonate can be replaced by sodium carbonate, but not by strong alkali such as potassium or sodium hydroxide, because these strong alkalis do the cleavage of the ether linkage between steroid and 2-benzoyl-4-nitro-phenyl group. Potassium or sodium bicarbonate is enough for migration of this group, but the benzylation is very slow.
13) J.D. Loudon, J.R. Robertson, J.N. Watson, and S.D. Alton, *J. Chem. Soc.*, **1950**, 55.
14) For the numerous examples of Smiles rearrangement, see the recent review; W.E. Truce, E.M. Kleider, and W.W. Brand, *Organic Reaction*, **18**, 99 (1970).

no significant difference in the reactivity between two hydroxyl groups as described above. However, the piperidine cleavage of the benzylated product gave isomeric mono-benzyl ethers (XVI) and (XVII), contrary to our expectation, in the ratio of 1: 3.8. This result may be concluded as that the BNP-group migrated from C-3 position to more basic C-2 position by Smiles rearrangement and then benzylation occurred mainly at C-3 position to give a predominant product of the one isomer (XVII). This was supported by the following reverse reaction.

The isomeric BNP-ether (XIV) synthesized from (XXXVII) by the same procedure as described by Miyazaki, *et al.*[15] was also treated with benzyl chloride as in the case of above experiment, and followed piperidine cleavage to give a mixture of (XVI) and (XVII) in the ratio of 1: 4.1, almost same ratio obtained from another starting substrate (XII). Migration of BNP-group took place here, however, it is only less 20% from 2-hydroxyl group to adjacent 3-position. These experiments showed that the phenoxide anion typed (XXV) is stronger base than the another one (XXIII), and that the latter is formed as predominant amount from both starting material by base. Because these reactions proceed under two different reactions: migration and substitution, it is complex to know the real degree of the migration. In the following experiment, the migration rate was studied kinetically by the simple system.




XXIII XXIV XXV

Chart 3

Isomeric mono-BNP ethers (XII) and (XIV) were refluxed respectively in ethanol in the presence of only potassium carbonate. Aliquots of the reaction mixture were taken with time intervals to be stopped by neutrallizing with acid and each product thus obtained was then treated with diazomethane to give a mixture of isomeric mono-methyl ethers (XIX) and (XX) respectively. These were then cleavaged by piperidine to give a mixture of isomeric mono-methyl ethers of 2-hydroxyestradiol, (XXI) and (XXII). These mixtures were then separated carefully on alumina chromatography under Breuer's condition.[16] The example of this separation is shown in Fig. 1. Each amount of product by different reaction times is shown in Fig. 2, from which it is easy to understand that the migration took place in the first stage of the reaction and that reaction system becomes in equilibrium of the irreversible migration of BNP-group within 30 min, and also that the anion (XXIII) exists six times than the another one (XXV) in the period of equilibrium.

From these results, it is also concluded that the phenoxide anion (XXV) is more reactive than its isomer (XXIII) to which migration proceeded *via* Meisenheimer type intermediate (XXIV).[17] Although the preparation of the spiro type Meisenheimer complex (XXVI) of ethylene glycol with *meta*-dinitrobenzene was reported by Fendler, *et al.*,[18] no examples of catechol with *meta*-dinitrobenzene or its analogues were reported. The authors tried to obtain the complex of catechol and catechol estrogen with *meta*-dinitro- or poly-

15) M. Miyazaki and J. Fishman, *J. Org. Chem.*, 33, 662 (1968).
16) R. Knuppen and H. Breuer, *Z. Physiol. Chem.*, 346, 114 (1966).
17) See the recent review; M.J. Straus, *Chem. Rev.*, 70, 667 (1970).
18) E.J. Fendler, J.H. Fendler, W.E. Byrne, and C.E. Griffin, *J. Org. Chem.*, 33, 4141 (1968).

nitrobenzene in no success. It seems to be an interesting problem to prepare the Meisenheimer complex of 2-hydroxylated estrogens or unsymmetrically substituted catechols for the detailed studies of Smiles rearrangement which is now under investigation.

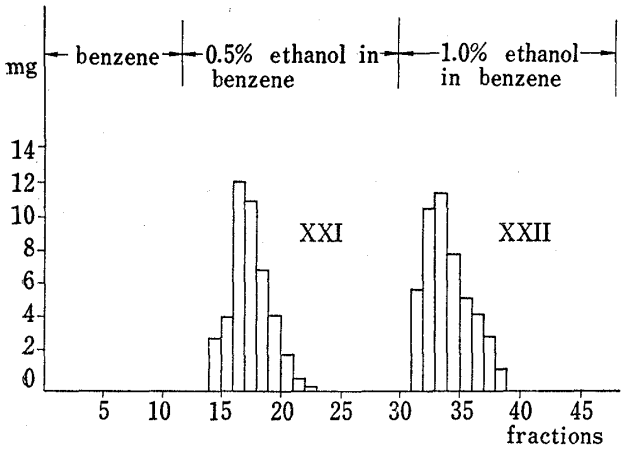



Fig. 1. Alumina Chromatography of Isomeric mono-Methyl Ethers of 2-Hydroxyestradiol obtained by Piperidine Cleavage of BNP-Ethers (XII) and (XIV) treated with Potassium Carbonate

Here, 2-piperidino-4-nitro-benzophenone was eluted in the fraction of *n*-hexane–benzene mixture (1:1).

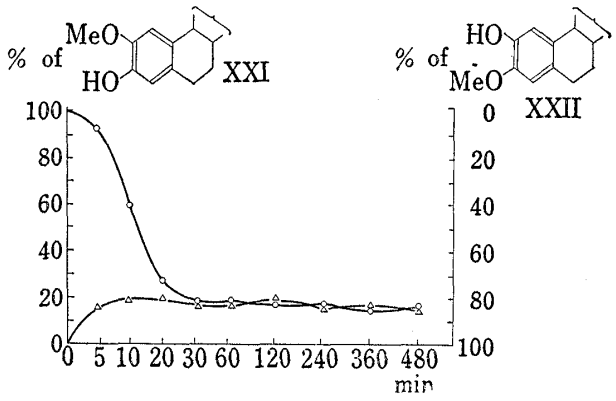
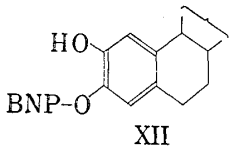


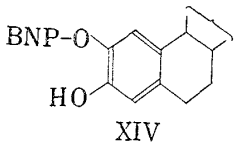

Fig. 2. Amounts of Isomeric mono-Methyl Ethers of 2-Hydroxyestradiol (XXI) and (XXII) derived from the Treatment of Isomeric BNP-Ethers(XII) and (XIV) with Potassium Carbonate at Various Reaction Times

—○—: from



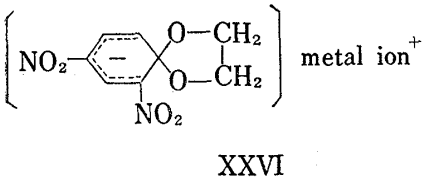
XII

—△—: from



XIV

The migration of BNP-group from weaker basic phenolate anion to the stronger one was first observed in the simple compound (XXVII), which was treated with base to be converted completely to another isomer (XXVIII) and no reverse migration proceeded.[13] In this unsymmetrical catechol derivative, the phenolate anion derived from the parent phenol to which BNP-group migrated is more basic than the original one due to the electron releasing effect of two methyl groups of the aromatic ring. Similarly the migration of benzoyl group of compound (XXIX) to adjacent phenolic group to give an isomer (XXX) is the same example.[19]




XXVI

Chart 4

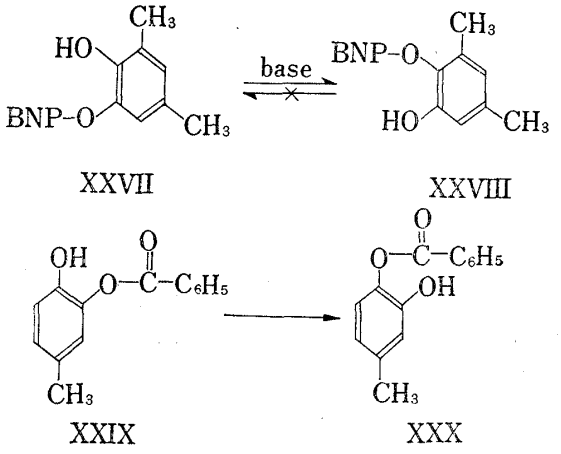


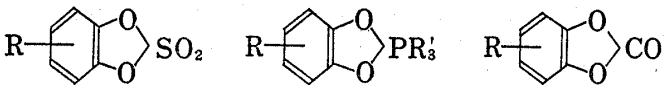

Chart 5

19) D.B. Denney and D.Z. Denney, *J. Am. Chem. Soc.*, **82**, 1389 (1960).

The difference of the results obtained by two experiments carried out with or without benzyl chloride are explained by the following reason: as the anion (XXV) is more basic than the isomer (XXIII), benzylation should proceed by different rate so that the equilibrium relation between (XXIII) and (XXV) is disturbed resulting some shift of the former to the latter *via* intermediate (XXIV) to give the resultant ratio of the two isomers.

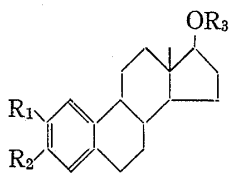
Similarly, the following five membered heterocyclic derivatives of catechols; cyclic sulfates (XXXI),[20] oxyphosphoranes (XXXII),[21] and *ortho*-phenylene-carbonates (XXXIII)[22] are interesting compounds in that which sulfur-, phosphorous-, and carbon-oxygen bonds are first cleavaged by the effect of substituent on catechol molecules.

The reason why the greater reactivity difference of the two hydroxyl groups of catechol estrogen was observed in the migration of BNP-group remains as an interesting problem. The possible reason may be that: as the carbon atom of BNP-group attached to steroid molecule *via* ether linkage is positively charged by two electron attracting groups, the phenoxide anion of (XXIII) or (XXV) attack(s) this position to give an intermediate (XXIV), but the essentially smaller difference between two anions appeared in farely greater amplitude in this attacking stage. If this is true, both anions (XXIII) and (XXV) formed by weak base should show some difference in the reaction activity. This was supported indirectly by the following experiment.



XXXI XXXII XXXIII

Chart 6



XXXIV: $R_1$=H, $R_2$=H, $R_3$=H
XXXV: $R_1$=$CH_3$-CO-, $R_2$=H, $R_3$=$CH_3$-CO-
XXXVI: $R_1$=H, $R_2$=$CH_3$-CO-, $R_3$=$CH_3$-CO-
XXXVII: $R_1$=OH, $R_2$=H, $R_3$=H

Chart 7

2-Hydroxyestrone (I) was treated with benzyl chloride and sodium bicarbonate instead of potassium carbonate. Two kinds of mono-benzyl ethers (IV) and (V) were obtained in the ratio of 2—2.5: 1 without formation of di-benzyl ether (VI). The formation of 2-benzyl derivative (IV) is twice as in the experiment in which potassium carbonate was used as a base. This result may indicate that the difference of the nucleophilicity of C-2 and C-3 phenolic anion was magnified by weaker base resulting a slightly predominant formation of 2-benzyl ether (IV).

It is noteworthy that phenolate anion of C-2 position is more basic than the C-3 position. This difference might be due to the result derived from the difference in electron density between C-2 and C-3 position of the aromatic ring. The fact that there is such a difference between these positions is shown in the Friedel–Craft acylation of 3-deoxy estradiol (XXXIV) to give 2-acylderivative (XXXV) in surprisingly exceeding amount with negligible another isomer (XXXVI).[23,24] Also the fact that C-1 proton of catechol estrogen resonates in down field than C-4 proton[24,25] may be attributed to the above distribution of electron density

20) G. Tomalin, M. Trifunac, and E.T. Kaiser, *J. Am. Chem. Soc.*, **91**, 722 (1969).
21) F. Ramirez, *Acounts of Chem. Res.*, **1**, 168 (1968); F. Ramirez, C.P. Smith, J.F. Pilot, and A.S. Gulati, *J. Org. Chem.*, **33**, 3787 (1968).
22) H. Gross, J. Rusche, and M. Mirsch, *Ber.*, **96**, 1382 (1963); T.H. Fyfe and D.M. McMahon, *J. Org. Chem.*, **35**, 3699 (1970).
23) K. Sakakibara, M. Sawai, and K. Chuma, Japan Patent 9279 (1963); [*C.A.*, **59**, 14060 (1963)].
24) T. Nambara, S. Honma, and S. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 474 (1970).
25) J. Fishman and J.S. Liang, *Tetrahedron*, **24**, 2199 (1968).

on aromatic ring as well as the sterical environment of estrogen molecule as described by Fishman, *et al.*[25]

In the result, the chemical difference of two phenolic hydroxyl groups of catechol estrogen is not remarkable one from our present results. This difference, however, might be so great for enzyme itself in the living animals resulting to give a selective biotransformation as cited above.

## Experimental[26]

**Benzylation of 2,3-Dihydroxyestra-1,3,5(10)-trien-17-one (I)**——(A) With $K_2CO_3$: The titled compound (988 mg, 2.94 mmole) was dissolved in 50 ml ethanol containing benzyl chloride (360 mg, 2.85 mmole) and $K_2CO_3$ (2.20 g). This solution was refluxed for 4 hr under nitrogen stream. After the reaction mixture was cooled, ethanol was removed under reduced pressure until about 10 ml ethanol remained. To this solution, benzene (200 ml) was added and this was washed with 1N HCl and then with water and finally dried over $Na_2SO_4$. Removal of the solvent gave a syrup (996 mg). This product was then adsorbed on a neutral alumina (100 g) which was developed with *n*-hexane–benzene–ethanol system.

Elution with *n*-hexane–benzene (1:1) gave material (77 mg), which was recrystallized from *n*-hexane to give a fine needless, mp 163—163.5, and this compound was identified as 2,3-dibenzyloxyestra-1,3,5(10)-trien-17-one (VI). *Anal.* Calcd. for $C_{32}H_{34}O_3$: C, 82.37; H, 7.35. Found: C, 82.46; H, 7.43. NMR (in $CDCl_3$) $\delta$: 7.45—7.35 (10H, multiplet, $2C_6H_5$), 6.88 (1H, singlet, aromatic $C_1$-H), 6.62 (1H, singlet, aromatic $C_4$-H), 5.02 (4H, singlet, $C_6H_5$-C$\underline{H}_2$), 0.89 (3H, singlet, 18-$CH_3$). IR: $\nu_{max}^{Nujol}$; 1732 $cm^{-1}$ (17>CO).

Elution with benzene gave material (464 mg) which was recrystallized from ethanol to give a fine needless, mp 193—194°. This was assigned as 2-benzyloxy-3-hydroxyestra-1,3,5(10)-trien-17-one (IV). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.60; H, 7.44. NMR (in $CDCl_3$) $\delta$: 7.45—7.35 (5H, multiplet, $C_6H_5$), 6.83 (1H, singlet, aromatic $C_1$-H), 6.63 (1H, singlet, aromatic $C_4$-H), 5.03 (2H, singlet, $C_6H_5$-C$\underline{H}_2$-), 0.89 (3H, singlet, 18-$CH_3$). IR: $\nu_{max}^{Nujol}$; 1734 $cm^{-1}$ (17>CO).

Elution with 1.0% ethanol in benzene gave material (378 mg), which was recrystallized from acetone to give a fine needless, mp 221—221.5. This material was assigned as 2-hydroxy-3-benzyloxyestra-1,3,5(10)-trien-17-one (V). *Anal.* Calcd. for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.56; H, 7.39. NMR (in $CDCl_3$) $\delta$: 7.45—7.35 (5H, multiplet, $C_6H_5$), 6.89 (1H, singlet, aromatic $C_1$-H), 6.67 (1H, singlet, aromatic $C_4$-H), 5.05 (2H, singlet, $C_6H_5$-C$\underline{H}_2$-), 0.90 (3H, singlet, 18-$CH_3$). IR: $\nu_{max}^{Nujol}$; 1734 $cm^{-1}$ (17>CO).

(B) With $NaHCO_3$: Same substrate (430 mg, 1.50 mmole) was refluxed in 30 ml ethanol containing a benzyl chloride (183 mg, 1.40 mmole) and $NaHCO_3$ (980 mg) for 40 hr under nitrogen stream. Two monobenzyl ethers (IV) and (V) were obtained by the same procedure described above. Here, dibenzyl ether (VI) was not obtained enough to recrystallize. Amounts of each mono-benzyl ether were: (IV); 306 mg and (V); 153 mg.

**Acetylation of 2,3-Dihydroxyestra-1,3,5(10)-trien-17-one (I)**——To a solution of pyridine (5 ml) containing titled compound (180 mg, 0.63 mmole) was added an acetic anhydride (0.65 mmole). This mixture was then allowed to stand for 24 hr at room temperature and then powered onto ice water, then extracted with benzene. The extract was washed with 0.1 N $Na_2CO_3$ and with 0.1 N HCl, and finally with water and dried over $Na_2SO_4$. Evaporation of solvent under reduced pressure gave a residue. NMR spectrum of this extract showed a presence of two acetates of 2-hydroxyestrone. The ratio of 2-acetate to 3-acetate was about 1.2:1 by peak area. NMR (in $CDCl_3$) $\delta$: 6.93 ($C_1$-H of two isomers), 6.75 ($C_4$-H of 3-acetate), 6.64 ($C_4$-H of 2-acetate). For more details, this mixture was then treated with $CH_2N_2$ in ether for over night at 4°. No starting material was detected on thin-layer chromatography (TLC) plate by this condition. The material obtained by removal of ether was then separated by preparative TLC by multiple running in the mixture of *n*-hexane–benzene (4:1).

From the upper zone, 2-methoxy-3-acetoxyestra-1,3,5(10)-trien-17-one (X) was obtained (79 mg), which was recrystallized from methanol to give a fine needless, mp 152—153°. *Anal.* Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.65; H, 7.61. NMR (in $CDCl_3$) $\delta$: 6.86 (1H, singlet, aromatic $C_1$-H), 6.70 (1H, singlet, aromatic $C_4$-H), 3.78 (3H, $OCH_3$), 2.28 (3H, singlet, $OCOCH_3$), 0.89 (3H, singlet, 18-$CH_3$).

From the lower zone, material (103 mg) was obtained and this was assigned as 2-acetoxy-3-methoxyestra-1,3,5(10)-trien-17-one (IX), which was recrystallized from methanol to give a fine needless, mp 169—170°. *Anal.* Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.39; H, 7.62. NMR (in $CDCl_3$) $\delta$: 6.83 (1H, singlet, aromatic $C_1$-H), 6.57 (1H, singlet, aromatic $C_4$-H), 3.76 (3H, singlet, $OCH_3$), 2.26 (3H, singlet, $OCOCH_3$), 0.90 (3H, singlet, 18-$CH_3$).

26) Melting points were determined on a micro hot-stage and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Japan Electron Optics Lab. JMN-3H-60 spectrometer by using 5% solutions containing tetramethylsilane as an internal standard. The infrared spectra were taken with Nihon Bunko Koken-DS-301.

**Benzylation of 2-Hydroxy-17$\beta$-acetoxyestra-1,3,5(10)-trien-3-(2-benzoyl-4-nitro)-phenyl Ether (XII)** Titled compound (865 mg, 1.56 mmole) was dissolved in 50 ml ethanol containing benzyl chloride (201 mg, 1.60 mmole) and $K_2CO_3$ (1.0 g), and this mixture was refluxed for 8 hr and after this reaction mixture was cooled, ethanol was removed under reduced pressure until about 10 ml of solvent remained. To this solution, $CHCl_3$ was added and this solution was washed with water and then dried over $Na_2SO_4$. Evaporation of chloroform gave a syrup (980 mg), which was dissolved in 50 ml of piperidine and refluxed for 30 min. After the reaction mixture was cooled, 1N HCl was added to the solution for neutrallizing the basic solvent, and then extracted with benzene. The extract was washed with water and then dried over $Na_2SO_4$. Evaporation of benzene gave yellow syrup which was submitted to alumina chromatography.

From *n*-hexane–benzene mixture (1:1), 2-piperidino-4-nitrobenzophenone (245 mg) was obtained as yellow syrup.

From benzene fraction, fine crystals were obtained in small amount (20 mg), mp 115—117°. This was identified as 2,3-dibenzyloxyestra-1,3,5(10)-trien-17$\beta$-ol (XVIII). *Anal.* Calcd. for $C_{32}H_{36}O_3$: C, 82.01; H, 7.74. Found: C, 79.94; H, 7.68. NMR (in DMSO-$d_6$) $\delta$: 7.45—7.35 (10H, multiplet, 2 $C_6H_5$), 6.96 (1H, singlet, aromatic $C_1$-H), 6.76 (1H, singlet, aromatic $C_4$-H), 5.06 (4H, singlet, $C_6H_5$-C$\underline{H}_2$-), 0.66 (3H, singlet, 18-$CH_3$).

From the fraction of 1.0% ethanol in benzene, material (163 mg) was obtained and this was recrystallized from $CHCl_3$ and *n*-hexane mixture to give a fine needless, mp 180.5—181.5. This compound was identified as 2-benzyloxyestra-1,3,5(10)-trien-3,17$\beta$-diol (XVI). *Anal.* Calcd. for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00. Found: C, 80.11; H, 7.03. NMR (in DMSO-$d_6$) $\delta$: 7.45—7.35 (5H, multiplet, $C_6H_5$), 6.84 (1H, singlet, aromatic $C_1$-H), 6.50 (1H, singlet, atomatic $C_4$-H), 5.05 (2H, singlet, $C_6H_5$-C$\underline{H}_2$-), 0.66 (3H, singlet, 18-$CH_3$).

From 2—4% ethanol in benzene fraction, material (541 mg) was obtained. This was recrystallized from ethanol to give a fine needless, mp 229.5—230.5, and this was identified as 3-benzyloxyestra-1,3,5(10)-trien-2,17$\beta$-diol (XVII). *Anal.* Calcd. for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00. Found: C, 80.02; H, 6.93. NMR (in DMSO-$d_6$) $\delta$; 7.45—7.35 (5H, multiplet, $C_6H_5$), 6.71 (1H, singlet, aromatic $C_1$-1), 6.62 (1H, singlet, aromatic $C_4$-H), 5.02 (2H, singlet, $C_6H_5$-C$\underline{H}_2$-), 0.66 (3H, singlet, 18-$CH_3$).

**Benzylation of 2-Hydroxy-17$\beta$-acetoxyestra-1,3,5(10)-trien-2-(2-benzoyl-4-nitro)-phenyl Ether (XIV)** The above titled compound (490 mg, 0.89 mmole) was dissolved in 40 ml ethanol containing benzyl chloride (113 mg, 0.90 mmole) and $K_2CO_3$ (460 mg). The condition and procedure were completly same as in the experiments in the above section. Finally, dibenzyl ether (XVIII), 2-benzyl derivative (XVI), and 3-benzyl derivative (XVII) were obtained as in the following amounts; 4 mg, 93 mg, and 343 mg respectively.

**Kinetical Experimentals**——(A) 2-Hydroxy-17$\beta$-acetoxyestra-1,3,5(10)-trien-3-(2-benzoyl-4-nitro)phenyl Ether (XII): The titled compound (1.40 g) was dissolved in 250 ml ethanol containing 2.0 g of $K_2CO_3$ and this mixture was refluxed. 25 ml of reaction mixture was taken out after 5,10,20,30,60,120,240,360, and 480 min and then each aliquot was acidified by 0.5 N HCl (50 ml). These were then extracted with benzene and extract was washed with water. Evaporation of solvent under reduced pressure and residue obtained was dried well. Each product thus obtained was then treated with excess $CH_2N_2$ in ether (50 ml) and stored in refrigirator for over night. On TLC of silica gel in 10% acetone in benzene, no starting material was observed by this condition. Then ether was removed to give an oil, which was then cleavaged by piperidine (20 ml) by refluxing for 30 min. Neutralization by 1N HCl of piperidine was carried out and followed an extraction of materials by benzene. Extract was then washed with water and repeated drying with benzene–ethanol mixture to give a syrup. The product thus obtained was then adsorbed on a neutral alumina column which was developed with benzene and benzene–ethanol mixture. Fractions of 5 ml were collected and each was weighed after evaporation of solvent. The actual distribution of materials between the two isomers were obtained by combining the fractions each (Fig. 1). The identity of each was confirmed by melting point and NMR spectroscopy.

(B) 2-Hydroxy-17$\beta$-acetoxyestra-1,3,5(10)-trien-2-(2-benzoyl-4-nitro)-phenyl Ether (XIV): Completly same procedure was carried out by using 1.24 g of the titled compound in 250 ml ethanol containing $K_2CO_3$ (1.75 g). The results obtained are shown in Fig. 2.