The authentic sample was prepared by the method described by Brown[6] and Djerassi[5] from cholestenone. Our obtained 4α-ethylcholestan-3-one showed the following physical constants. m.p. 120~121°, $[\alpha]_D^{21}$ +36.0±2°(c=0.918, $CHCl_3$).

4α-Ethylcholestane (XI)——The ketone (44 mg.) obtained from the desulfurization product of VII was treated with 0.2 g. of KOH, 0.4 cc. of $NH_2NH_2 \cdot H_2O$, and 4 cc. of triethyleneglycol in the same manner as for the preparation of VIII. The product was purified by passing through a column of 1 g. of Al_2O_3 in a petr. ether solution, and was recrystallized from Et_2O-MeOH to give 30 mg. of needles, m.p. 86~88°. $[\alpha]_D^{24}$ +10.9±4°(c=0.594, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{52}$: C, 86.92; H, 13.08. Found : C, 87.19; H, 13.11.

This compound was identified as 4α-ethylcholestane by mixed melting point and comparison of the IR spectrum with that of the authentic sample. The authentic sample was prepared by the Huang-Minlon reduction of 4α-ethylcholestan-3-one prepared from cholestenone and showed the following physical constants. m.p. 86~88°. $[\alpha]_D^{24}$ +12.3±2°(c=1.058, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{52}$: C, 86.92; H, 13.08. Found : C, 86.64; H, 12.94. Reported by Brown, m.p. 89~90°, $[\alpha]_D$ +4.5°, +6°.

Summary

The 3,3-ethylenedioxy-5α,6α-epoxy steroids were converted to the 5α-hydroxy-6β-acetylthio-3-oxo steroids by the action of thiolacetic acid. These were dehydrated, and following by epimerization of the substituent at position 6 converted to 6α-acetylthio-4-en-3-one steroids. The thiophene derivatives were obtained from the last mentioned compounds by the action of basic alumina.

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91. Kiyoshi Tsuneda, Joji Yamada, Kikuo Yasuda, and Hiromu Mori : Preparation of Some Estriol Esters.

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In some cases esters of steroidal hormones show long-acting hormonal activities compared with the parent hormones. In the hope of preparing long-acting steroidal hormones, many esters of steroidal hormones such as testosterone, estradiol and others, have been prepared and then activities have been studied.[1] Nevertheless, no estriol ester, except the triacetate[2] and 3,16-diacetate[3] has beenpre pared. It is interesting to prepare various estriol esters and study about their activities. In the present paper, the preparation of some esters of estriol will be described.

In 1956 it was reported from this laboratory that estradiol is acylated only at C-17-hydroxyl group by reflux with a carboxylic acid[4] or by transesterification[5] to give estradiol 17-monoacylate. When estriol (I) was treated with acetic acid or with methyl acetate (transesterification), in the same way, a material (IIa), m.p. 190~193°, was obtained. Elemental analysis showed it is an estriol monoacetate. In order to deter-

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[1] K. Junkmann, H. Witzel : Zeitschrift für Vitamin-, Hormon- und Fermentforschung, **9**, 98, 227 (1957).

[2] A. Butenandt, E. Schäffler : Z. Naturforschung, **1**, 82 (1946).

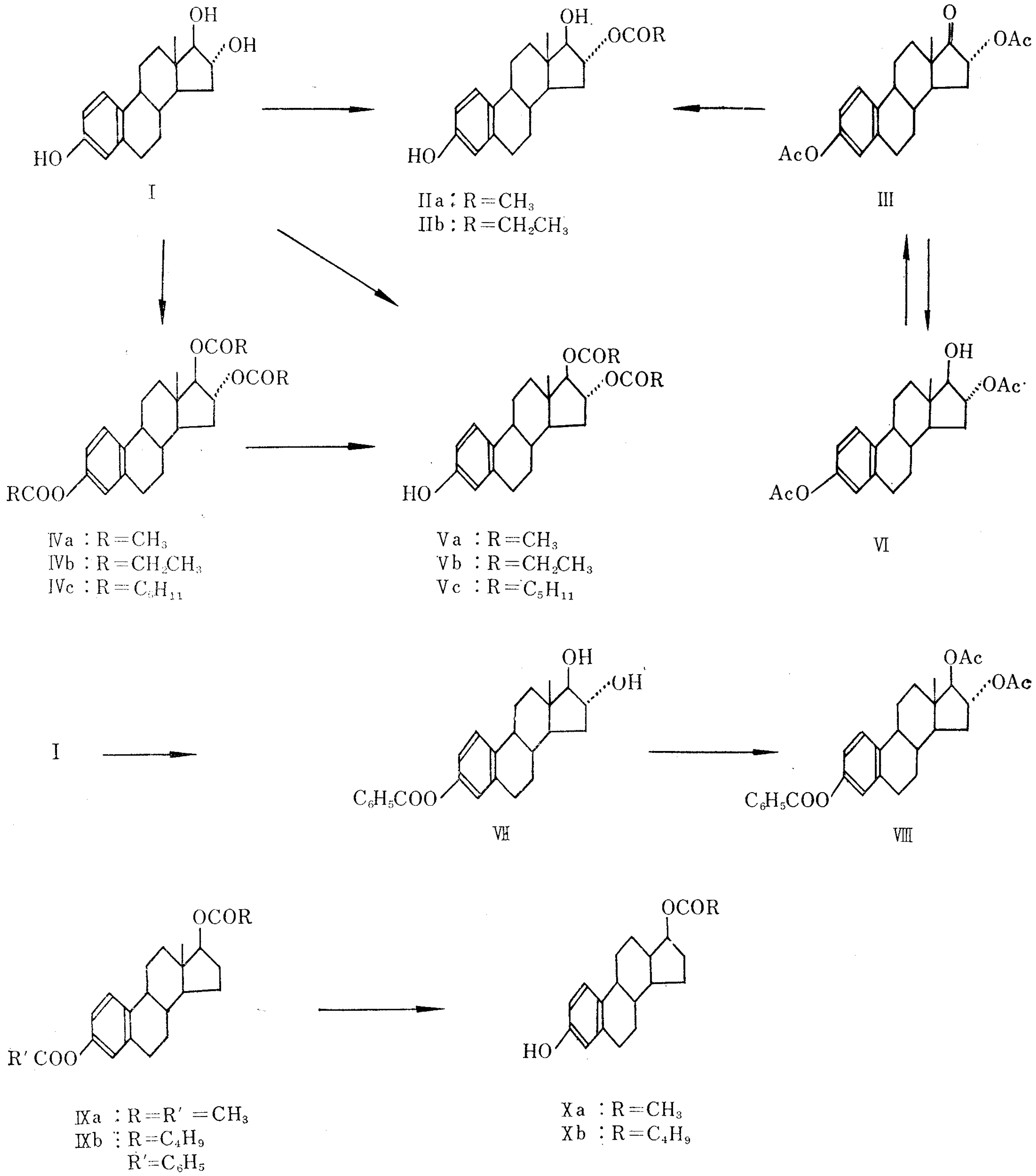
[3] J. Fishman : J. Am. Chem. Soc., **82**, 6143 (1960).

[4] M. Hosoi, H. Mori : Yakugaku Zasshi, **76**, 847 (1956).

[5] *Idem* : *Ibid.*, **76**, 849 (1956).

mine which hydroxyl group had been acetylated, 3,16-dihydroxyestra-1,3,5(10)-trien-17-one diacetate (Ⅲ) was reduced with an excess of sodium borohydride at room temperature, and the same acetate (Ⅱa) was obtained in a good yield. When one equivalent of sodium borohydride was used, estriol 3,16-diacetate (Ⅵ) was obtained. Accordingly, the monoacetate (Ⅱa) should be either the 3-acetate or the 16-acetate of estriol, but the structure of 3-acetate is unlikely, because the reaction of estradiol diacetate (ⅩⅠa) with sodium borohydride afforded estradiol 17-acetate (Ⅹa). Thus the substance (Ⅱa) can be formulated as estriol 16-acetate.

Recently, Neeman and Hashimoto[6] isolated estriol monoglucuronate from urine, and



I

Ⅱa : R=CH_3
Ⅱb : R=CH_2CH_3

Ⅲ

Ⅳa : R=CH_3
Ⅳb : R=CH_2CH_3
Ⅳc : R=C_5H_{11}

Ⅴa : R=CH_3
Ⅴb : R=CH_2CH_3
Ⅴc : R=C_5H_{11}

Ⅵ

Ⅶ

Ⅷ

Ⅸa : R=R′=CH_3
Ⅸb : R=C_4H_9
R′=C_6H_5

Ⅹa : R=CH_3
Ⅹb : R=C_4H_9

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[6] M. Neeman, Y. Hashimoto : **Tetrahedron** Letters, 183 (1961).

determined its structure as estriol 16-glucuronate. It is worthy to note that the C-16-hydroxyl group in estriol is the most easily acylated both biologically and chemically.

The present observation suggests that a selective fission of the acyl group in phenolic steroids is possible in general with sodium borohydride. In fact, estradiol 3-benzoate 17-valerate (IXb) was converted into estradiol 17-valerate (Xb) by this method. When estriol triacylates, IVa, IVb, and IVc were treated with sodium borohydride, estriol 16,17-diacylates, Va, Vb and Vc, were obtained in good yields.

The acylation of estriol with propionic acid and with ethyl propionate, was also tried and, in contrast to the above-mentioned case of acetylation, estriol 16,17-dipropionate (Vb) was obtained in either case. It seemed that the reaction condition was so drastic that the dipropionate (Vb) was given. A controlled transesterification with methyl propionate produced estriol 16-propionate (IIb). On the other hand, a more drastic acetylation of estriol at 150° in a sealed tube gave estriol 16,17-diacetate (Va).

Benzoylation of estriol (I) by Schotten-Baumann method gave estriol 3-benzoate (VII), which was acetylated with acetic anhydride in pyridine to give estriol 3-benzoate 16,17-diacetate (VIII). The same substance VIII was also isolated from the reaction product of transesterification of estriol 3-benzoate (VII).

Among these estriol esters, estriol 3-benzoate 16,17-diacetate (VIII), tripropionate (IVa) and 16,17-dipropionate (Va) showed long-acting estrogenic activities.[7] The detail of biological properties of these esters will be described elsewhere.

Experimental*[2]

Estriol 16-Acetate (IIa). A) By Acetylation of Estriol with Acetic Acid——Estriol (I, 500 mg.) was refluxed with AcOH (25 ml.) for 4hr. The reaction mixture was poured into H_2O, the product was extracted with Et_2O and the ethereal extract was washed with H_2O, 5% Na_2CO_3, and H_2O. After drying over Na_2SO_4, the solvent was evaporated, and the residue was recrystallized from Me_2CO-hexane mixture to give crude estriol 16-acetate (IIa, 200 mg., m.p. 182~186°). Three recrystallizations from the same solvent gave an analytical sample in colorless plates, m.p. 191~193°, $[\alpha]_D^{20}$: +38° (c=0.6, dioxane). *Anal.* Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found : C, 72.65; H, 8.08.

B) By Transesterification——A solution of estriol (I, 500 mg.) and *p*-toluenesulfonic acid monohydrate (500 mg.) in methyl acetate (30 ml.) was distilled slowly. During the distillation methyl acetate was added to keep the volume of the solution unchanged. After 3 hr distillation, Et_2O was added and the ethereal solution was washed with H_2O and dried over Na_2SO_4. The residue was recrystallized from Me_2CO-hexane mixture to give the same substance as mentioned above (310 mg., m.p. 186~191°).

C) From 3,16-Dihydroxyestra-1,3,5(10)-trien-17-one Diacetate (III)——3,16-Dihydoxyestra-1,3,5(10)-trien-17-one (III, 500 mg.) was dissolved in MeOH (40 ml.), and $NaBH_4$ (500 mg.) was added. The solution was stirred at room temperature for 45 min., and Me_2CO was added to decompose the excess of $NaBH_4$. The solution was poured into H_2O, the product was extracted with Et_2O, and the ethereal extract was washed with H_2O. After drying over Na_2SO_4, the solvent was evaporated. The residue was recrystallized from Me_2CO-hexane mixture to give estriol 16-acetate (IIa, 225 mg., m.p. 185~190°). It was identical with the material obtained above in all respects.

Estriol 16,17-Diacetate (Va). A) From Estriol Triacetate (IVa)——Estriol triacetate (IVa, 1.0 g.) was dissolved in MeOH (40 ml.) and $NaBH_4$ (250 mg.) was added. The solution was stirred for 1 hr. and AcOH was added to decompose the excess of $NaBH_4$. The solution was poured into water, the product was extracted with Et_2O, and the ethereal solution washed with 5% Na_2CO_3 and H_2O. After drying over Na_2SO_4, the solvent was evaporated and the residue was recrystallized from benzene-hexane mixture to give crude estriol 16,17-diacetate (500 mg., m.p. 168~171°). Further recrystallization from the same solvent gave an analytical sample in colorless prisms, m.p. 171~173°, $[\alpha]_D^{20}$: −19° (c=0.94, dioxane). *Anal.* Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found : C, 71.47; H, 7.70.

B) By the Acetylation of Estriol (I) with Acetic Acid——A solution of estriol (I, 1.0 g.) in AcOH (50 ml.) was heated to 150° in a sealed tube for 4 hr., and poured into H_2O. The product was extracted with Et_2O and the ethereal solution was washed with H_2O, 5% Na_2CO_3 and H_2O. After drying over Na_2SO_4, the solvent was evaporated. The residue was recrystallized from Et_2O-petr. ether mixture to give estriol 16,17-diacetate (Va, 510 mg., m.p. 167~172°) identical with the material obtained above.

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*[2] All melting points are uncorrected.

[7] H. Ando *et al.* : Unpublished data.

Estriol 3,16-Diacetate (VI)——A solution of 3,16α-dihydroxyestra-1,3,5(10)-trien-17-one diacetate (III, 200 mg.)[8] in dioxane (3.0 ml.) and MeOH (16 ml.) was cooled in ice-cold H_2O and $NaBH_4$(14 mg.) was added. The solution was stirred for 25 min. After addition of AcOH, the solution was poured into H_2O, the product was extracted with Et_2O, and the ethereal extract was washed with 5% Na_2CO_3 and HO_2 and dried over Na_2SO_4. The solvent was evaporated and the residue was recrystallized from Et_2O-petr. ether to give estriol 3,16-diacetate (105 mg., m.p. 150~152°). $[\alpha]_D^{20}$: +113°(c=0.51, $CHCl_3$), $[\alpha]_D^{19}$: +32° (c=1.1, dioxane). *Anal.* Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 70.78; H, 7.72.

Estriol 3-Benzoate (VII)——A solution of estriol (500 mg.) in 2% NaOH (200 ml.) was shaken vigorously with benzoyl chloride (3.5 ml.) in a separating funnel for 30 min. The resulting precipitates were collected by filtration, washed with H_2O, dried, and recrystallized from Me_2CO to givo estriol 3-benzoate (VII, 300 mg., m.p. 214~224°). Further recrystallization from Me_2CO gave an analytical sample, m.p. 226~229°, $[\alpha]_D^{20}$: +34°(c=1.1, dioxane). *Anal.* Calcd. for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.17; H, 7.08.

Estriol 3-Benzoate 16,17-Diacetate (VIII). A) By Acetylation——Estriol 3-benzoate (VII, 250 mg.) was dissolved in pyridine (2.5 ml.) and Ac_2O (5.0 ml.), and the mixture was kept at room temperature overnight. The solution was poured into H_2O, the product was extracted with Et_2O, and the ethereal solution was washed with 10% HCl, 5% Na_2CO_3 and H_2O and dried over Na_2SO_4. After evaporation of the solvent the residue was recrystallized from MeOH to give estriol 3-benzoate 16,17-diacetate (VIII, 170 mg.) in colorless plates. m.p. 180~182°, $[\alpha]_D^{20}$: −2°(c=0.92, dioxane). *Anal.* Calcd. for $C_{29}H_{32}O_6$: C, 72.86; H, 6.77. Found: C, 72.86; H, 6.79.

B) **By Transesterification**——Estriol 3-benzoate (500 mg.) was treated with methyl acetate (30 ml.) and *p*-toluenesulfonic acid monohydrate (500 mg.) as described above. Estriol 3-benzoate 16,17-diacetate (m.p. 179~181°) was obtained.

Estriol Tripropionate (IVb)——Estriol (1.0 g.) was dissolved in pyridine (5.0 ml.) and propionic anhydride (5.0 ml.) and the solution was stored at room temperature overnight. The solution was poured into H_2O, the product was extracted with Et_2O, and the extract was washed with 10% HCl, 5% Na_2CO_3 and H_2O and dried over Na_2SO_4. The evaporation of the solvent afforded a yellow gum, which on recrystallization from hexane gave estriol tripropionate (IVb, 0.96 g., m.p. 99~100°). Further recrystallization from hexane gave an analytical sample in colorless cubes, m.p. 100~101°, $[\alpha]_D^{20}$: −9°(c=1.02, dioxane). *Anal.* Calcd. for $C_{27}H_{36}O_6$: C, 71.02; H, 7.95. Found: C, 71.08; H, 8.07.

Estriol 16,17-Dipropionate (Vb). A) From Estriol Tripropionate (IVb)——$NaBH_4$(250 mg.) was added to a solution of estriol tripropionate (IVb, 1.0 g.) in MeOH (40 ml.) with stirring and cooling in ice H_2O. The solution was stirred for 1 hr. and poured into 2% AcOH. The product was extracted with Et_2O, and the extract was washed with 5% Na_2CO_3 and H_2O and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on Florisil, and estriol 16,17-dipropionate (Vb) was obtained from benzene eluate. It was recrystallized from Et_2O-petr. ether to give an analytical sample (620 mg.) in colorless needles. m.p. 127~130°, $[\alpha]_D^{20}$: −14° (c=0.99, dioxane). *Anal.* Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.68; H, 8.14.

B) **By Transesterification of Estriol (I)**——Estriol (I, 1.0 g.) was treated with ethyl propionate (60 ml.) and *p*-toluenesulfonic acid monohydrate (1.0 g.) described above and estriol dipropionate (m.p. 126~129°) was obtained.

C) **By Reflux with Propionic Acid**——Estriol (I, 1.0 g.) was treated with propionic acid (50 ml.) as described above, and estriol 16,17-dipropionate (m.p. 128~129°) was obtained.

Estriol 16-Propionate (IIb)——A solution of estriol (I, 1.0 g.) and *p*-toluenesulfonic acid monohydrate (1.0 g.) in methyl propionate (60 ml.) was distilled slowly for 1 hr., the volume being kept constant by adding methyl propionate. Et_2O was added, and the ethereal solution was washed with H_2O and dried over Na_2SO_4. The solvent was evaporated and the residue was recrystallized from Me_2CO-petr. ether to give estriol 16-propionate (IIb, 520 mg., m.p. 202~205°). Further recrystallization from the same solvent afforded an analytical sample in colorless cubes, m.p. 205~207°, $[\alpha]_D^{20}$: 27°(c=1.19, dioxane). *Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.02; H, 8.34.

Estriol Tricaproate (IVc)——A solution of estriol (I, 1.0 g.) in pyridine (5.0 ml.) and caproic anhydride (2.5 ml.) was stored at room temperature overnight, and poured into H_2O. The product was extracted with Et_2O, and the ethereal extract was washed with 10% HCl, 5% Na_2CO_3 and H_2O. After drying over Na_2SO_4, the solvent was evaporated to give a yellow gum. It was chromatographed on alumina, and a colorless oily product (900 mg.) was obtained. Although it failed to crystallize, it was analytically pure and IR spectrum showed it was estriol tricaproate (IVc). $[\alpha]_D^{20}$: −7° (c=0.97, dioxane). *Anal.* Calcd. for $C_{36}H_{54}O_6$: C, 74.19; H, 9.34. Found: C, 73.95; H, 9.48.

Estriol 16,17-Dicaproate (Vc)——To an ice-cold solution of estriol tricaproate (1.0 g.) in MeOH (40 ml.), $NaBH_4$(250 mg.) was added, and after 1 hr. stirring the solution was poured into 2% AcOH. The product was extracted with Et_2O, and the ethereal extract was washed with 5% Na_2CO_3 and H_2O and

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8) N.S. Leeds, D.K. Fukushima, T.F. Gallagher: J. Am. Chem. Soc., **76**, 2943 (1954).

dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed on Florisil. Estriol 16,17-dicaproate (Vc, 700 mg.) was obtained from Et_2O eluates as an oily substance. It failed to crystallize but it was analytically pure and IR spectrum showed that it was estriol 16,17-dicaproate (Vc). $[\alpha]_D^{20}$: −6°(c=0.84, dioxane). *Anal.* Calcd. for $C_{30}H_{44}O_5$: C, 74.34; H, 9.15. Found: C, 73.98; H, 9.24.

Estradiol 17-Acetate (Xa)——A solution of $NaBH_4$ (1.0 g.) in MeOH (24 ml.) was added to a solution of estradiol diacetate (IXa, 1.0 g.) in MeOH (80 ml.). The solution was stirred at 40° for 45 min., and poured into 2% AcOH. The product was extracted with Et_2O, and the ethereal extract was washed with 5% Na_2CO_3 and H_2O and dried over Na_2SO_4. The solvent was evaporated and the residue was recrystallized from MeOH to give estradiol 17-acetate (Xa, 850 mg., m.p. 212~215°), the IR spectrum of which was the same as that of authentic sample.

Estradiol 17-Valerate (Xb)——Estradiol 3-benzoate 17-valerate (IXb, 60 mg.) was treated with $NaBH_4$ as described above and estradiol 17-valerate (Xb, m.p. 142~144°) was obtained.

We are very grateful to Dr. I. Chuman, Director of this laboratory for valuable advices, and Dr. F. Ueno and Dr. H. Tajima for their encouragement throughout this work.

Summary

Estriol 16-monoacylates were obtained from estriol (I) by refluxing or warming estriol with carboxylic acids. A more drastic condition afforded estriol 16,17-diacylates. Transesterification gave the same results. The reactions of estriol triacylates and estradiol diacylates with sodium borohydride gave estriol 16,17-diacylates and estradiol 17-acylates, respectively. 3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one diacetate (III) was transformed into estriol 16-acetate (IIa) by treatment with sodium borohydride. A milder condition afforded estriol 3,16-diacetate (VI).

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92. **Toshihiko Okamoto and Hiroaki Takayama**: The Reaction of N-Alkoxypyridinium Derivatives. (6).[1,2] The Reaction of N-Methoxyquinolinium Salt with Ketones.

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Reactions of N-alkoxypyridinium salt with nucleophilic reagents have been reported by several authors. N-Alkoxypyridinium derivative gave picolinonitrile and isonicotinonitrile when it was reacted with cyanide ion.[3] This reaction also proceeds with quaternary salts of quinoline-,[3] isoquinoline-,[3] phenanthridine-[4] and quinine N,N′-dioxides.[5] Further, 2-alkylpyridine was obtained by the reaction of N-alkoxypyridinium salt and alkyl magnesium halogenide.[6] The mechanism of these reactions have been established to be as follows.

[*1] Hongo, Bunkyo-ku, Tokyo (岡本敏彦, 高山浩明).

1) Part (5): H. Tani: Yakugaku Zasshi, **81**, 182 (1961).

2) This paper constitute Part IV of a series entitled Reaction Mechanism in Aromatic Heterocyclic Compounds by T. Okamoto. Part III: This Bulletin, 8, 892 (1960).

3) T. Okamoto, H. Tani: This Bulletin, **7**, 130, 925 (1959); H, Tani: *Ibid.*, **7**, 930 (1959); W, E. Feely, E.M. Beavers: J. Am. Chem. Soc., **81**, 4004 (1959); Y. Kagawa: J. Biochem., **47**, 104 (1960).

4) E. Hayashi, Y. Hotta: Yakugaku Zasshi, **80**, 834 (1960).

5) T. Okamoto, H. Takayama: *Ibid.*, **82**, 1076 (1962).

6) O. Cervinka: Chem. & Ind. (London), 1482 (1960).