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The Synthesis and Zimmermann Reaction of Some Androsten-16-ones[1]

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The synthesis and Zimmermann reaction of some androsten-16-ones having double bond in ring A and/or B were described. These 16-oxosteroids showed weak Zimmermann color as compared with saturated androstan-16-one, probably due to long-range conformational effect. The structure of Zimmermann complex derived from 3β-hydroxyandrost-5-en-16-one was also discussed.

As a part of our program dealing with the mechanism of Zimmermann reaction we have investigated the dehydroisoandrosterone index of various oxosteroids.[3] It was thereby observed that estrone-16 exhibited significantly low value compared with estrone in spite of bearing active methylene groups, and this property was tentatively interpreted in terms of long-range conformational effect due to aromatic A-ring.[4-6] The present paper describes the synthesis and Zimmermann reaction of some androsten-16-ones having double bond in ring A and/or B and the structure of Zimmermann complex derived from 16-oxosteroid.

The initial project was focused on the preparation of 3β-hydroxyandrost-5-en-16-one (VIIb) through the two different routes. First, 5α,6β-dichloro-3β,16α-dihydroxyandrostan-17-one diacetate (II), which was obtained from 5α,6β-dichloro-3β-hydroxyandrostan-17-one acetate by way of 16-en-17-ol acetate (I) and its 16α,17α-epoxide by the method of Mori and his co-workers,[7] was submitted to ketol rearrangement with base.[8] Treatment of II with methanolic potassium hydroxide at room temperature followed by reacetylation furnished 5α,6β-dichloro-3β,17β-dihydroxyandrostan-16-one diacetate (III) in 43% yield. Assignment of the structure is unequivocal, since of four possible 16,17-ketols in C/D-*trans* steroids 17β-hydroxy-16-oxo derivative is the most stable one.[9] When III was refluxed in acetic acid-acetic anhydride with zinc dust, simultaneous removal of both 5,6-dichloro and 17β-acetoxy groups took place providing 3β-hydroxyandrost-5-en-16-one acetate (VIIa) in 34% yield. Alternatively, 3β,17β-dihydroxyandrost-5-en-16-one (VI) prepared from dehydroisoandrosterone enol diacetate (IV) by lead tetraacetate oxidation and subsequent ketol rearrangement, was subjected to reductive elimination of 17β-hydroxyl group. Refluxing of VI in acetic acid-acetic anhydride with zinc dust resulted in formation of VIIa in excellent yield. Thus the second route proved to be more advantageous for the preparation of VIIa starting from dehydroisoandrosterone. Hydrolysis of VIIa with methanolic potassium

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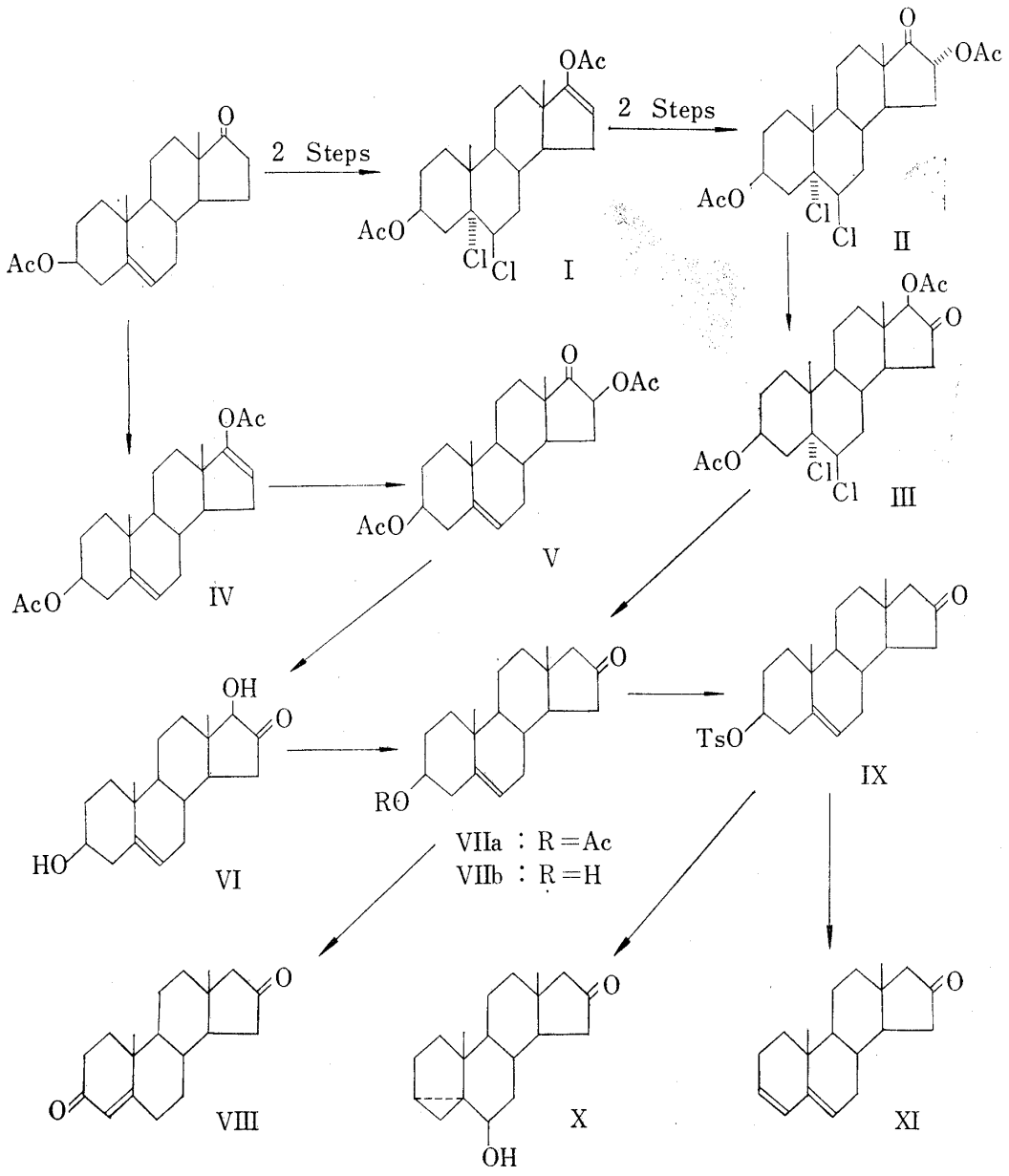
3) T. Nambara and M. Katō, *Chem. Pharm. Bull.* (Tokyo), **13**, 78 (1965).
4) D.H.R. Barton, *et al.*, *J. Chem. Soc.*, **1956**, 932; **1957**, 935; **1960**, 1297.
5) J. Fishman, *J. Am. Chem. Soc.*, **82**, 6143 (1960).
6) J. Fishman, *J. Org. Chem.*, **27**, 1745 (1962); **30**, 2841 (1965).
7) T. Aoki, H. Yamamura, K. Takei, and H. Mori, *Chem. Pharm. Bull.* (Tokyo), **12**, 808 (1964).
8) N.S. Leeds, D.K. Fukushima, and T.F. Gallagher, *J. Am. Chem. Soc.*, **76**, 2943 (1954).
9) J. Fishman, *J. Am. Chem. Soc.*, **82**, 6153 (1960).

hydroxide provided 3β–hydroxyandrost–5–en–16–one (VIIb), which in turn was oxidized with Jones reagnet[10] and then isomerized by contact with alumina to androst–4–ene–3,16–dione (VIII).

Now, the attempt was made on the synthesis of 6β–hydroxy–3α,5α–cycloandrostan–16–one (X) having *i*–structure with use of VIIb as the starting material. On usual treatment with *p*–toluenesulfonyl chloride in pyridine VIIb was transformed with ease into 3–tosylate (IX). When IX was refluxed with potassium acetate in aqueous acetone, the desired compound (X) was affored in 58% yield. In addition, androsta–3,5–dien–16–one (XI) was also prepared from IX by heating in dimethylsulfoxide[11,12] and subsequent usual purification.



Ac=CH_3CO-, Ts=$p-CH_3C_6H_4SO_2-$

Chart 1

10) C. Djerassi, R.R. Engel, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).
11) D.N. Jones and M.A. Saeed, *J. Chem. Soc.*, **1963**, 4657.
12) T. Nambara and M. Katō, *Chem. Pharm. Bull.* (Tokyo), **13**, 1435 (1965).

The Zimmermann reaction was carried out with these 16-oxosteroids according to the method of Callow, *et al.*,[13] and dehydroisoandrosterone indexes[3] were estimated in comparison with those of the related oxosteroids. All the compounds so far examined showed much smaller values than those of 3β-hydroxyandrostan-16-one as well as the corresponding 17-oxosteroids (see Table I). The drastic effect due to the structure alternation in distant ring is obviously recognized on the reactivity of 16-oxo function. It seems very likely that the long-range effect of estrone-16 previously observed would not be due to the lack of angular methyl group at C-10 but the presence of trigonal carbon at C-5 and/or C-10. No plausible explanation for the remote effect observed is now available. However, it should be emphasized that stereochemistry of ring D having oxo function at C-16 must be discussed with respect to the structure of ring A and/or B.

Table I. The Absorbance of the Colored Solution produced from 16- and 17-Oxosteroids by the Zimmermann Reaction

No.	Compounds	Concentration μM/0.2 ml	Absorbance at (mμ) 400	440	480	520	560	600	Dehydroisoandrosterone Index
0	3β-Hydroxyandrost-5-en-17-one (Dehydroisoandrosterone)	0.356	.178	.256	.422	.533	.430	.278	100
1	3α-Hydroxy-5α-androstan-17-one	0.331	.146	.206	.375	.493	.392	.260	99
2	3-Hydroxyestra-1,3,5(10)-trien-17-one	0.468	.240	.267	.535	.722	.604	.355	103
3	3β-Hydroxy-5α-androstan-16-one	0.411	.133	.203	.367	.495	.392	.227	81
4	Androst-4-ene-3,16-dione	0.710	.768	.598	.567	.641	.690	.583	60
5	3β-Hydroxyandrost-5-en-16-one	0.702	.206	.183	.183	.224	.237	.179	21
6	3β-Hydroxyandrost-5-en-16-one Acetate	0.655	.180	.176	.181	.214	.224	.158	22
7	3β-Hydroxyandrost-5-en-16-one Tosylate	0.426	.120	.107	.114	.152	.165	.111	24
8	6β-Hydroxy-3α,5α-cycloandrostan-16-one	0.707	.168	.149	.153	.192	.204	.150	18
9	Androsta-3,5-dien-16-one	0.830	.211	.189	.206	.273	.296	.198	22
10	3-Hydroxyestra-1,3,5(10)-trien-16-one Acetate	0.356	.101	.092	.099	.108	.104	.077	20

Next project was directed toward the isolation of Zimmermann complex of 3β-hydroxyandrost-5-en-16-one. The Zimmermann reaction was carried out on the preparative scale and the reaction mixture was chromatographed on acid-washed alumina followed by recrystallization of the eluate, hereupon, the expected Zimmermann complex, mp 220—224°, was isolated in a crystalline state with success. Inspection of NMR spectrum shows that dinitrophenyl group is attached to the steroid nucleus with *o,p*-position of nitro groups on the basis of following interpretation. As can be seen in Fig. 1, three groups of peaks in the range of 1—3 τ would be assigned to three protons on benzene ring, namely Ha (1.20 τ), Hb (1.60 τ) and Hc (2.44 τ), where Hb is split by coupling with Ha (J=3 cps) and Hc (J= 9 cps) to give quartet. These NMR patterns were observed common to all the Zimmermann complexes. Furthermore, it is also indicated that 2,4-dinitrophenyl group would be introduced to C-17 rather than C-15, since one proton signal appears at 5.58 τ as singlet. It is noteworthy that displacement of Zimmermann chromophore would take place in the same direction as enolization of 16-oxo group[14]. This fact might be a good explanation for the negative result of C-17-substituted 16-oxosteroid despite of having active methylene group.

13) N.H. Callow, R.K. Callow, and C.W. Emmens, *Biochem. J.*, **32**, 1312 (1938).

14) An analogous instance has also been observed on the Zimmermann reaction with cholestan-3-one, where the chromophore would be introduced toward the more readily enolizable direction in regard to 3-oxo group, that is C-2-position.[15]

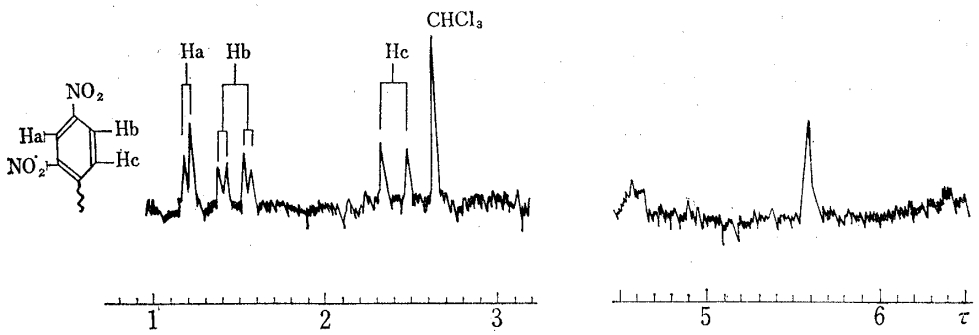


Fig. 1. The NMR Spectrum of the Zimmermann Complex of 3β-Hydroxyandrost-5-en-16-one

JNM 3H-60; 60 Mc; $CDCl_3$ Solution

Further studies on the mechanism of Zimmermann reaction in respect with stereochemistry of oxosteroids are being conducted in these laboratories and will be reported in near future.

Experimental[16]

5α,6β-Dichloro-3β,17-dihydroxyandrost-16-ene Diacetate (I)——To a solution of 5α,6β-dichloro-3β-hydroxyandrostan-17-one acetate (4.9 g) in isopropenyl acetate (35 ml) was added catalyst solution (5 ml of isopropenyl acetate and 0.1 ml of conc. H_2SO_4) (2.5 ml), the reaction mixture was refluxed for 1 hr and approximately 20 ml was distilled off for 1 hr. An additional isopropenyl acetate (18 ml) containing catalyst solution (1.25 ml) was added and concentrated to one-half of its volume by slow distillation over another 2 hr. The resulting mixture was diluted with ether, washed with ice-cooled 5% $NaHCO_3$, H_2O and dried over anhyd. Na_2SO_4. After evaporation of solvent the residue was dissolved in *n*-hexane and filtered through Al_2O_3 (8 g). Upon concentration of the filtrate the crystalline product was obtained. Recrystallization from MeOH gave I (3.45 g) as colorless leaflets. mp 161—163°, $[\alpha]_D^{31}$ −69.0° (c=0.71). *Anal.* Calcd. for $C_{23}H_{32}O_4Cl_2$: C, 62.44; H, 7.29. Found: C, 61.86; H, 7.07. (Reported mp 146—150°, $[\alpha]_D^{27}$ −22°).[5]

5α,6β-Dichloro-3β,17β-dihydroxyandrostan-16-one Diacetate (III)——A solution of 5α,6β-dichloro-3β,16α-dihydroxyandrostan-17-one diacetate (II) (1 g) dissolved in 0.04 N NaOH (60% aq. MeOH) (500 ml) was allowed to stand at room temperature for 4 hr. The reaction mixture was diluted with AcOEt, washed with 5% HCl, H_2O and dried over anhyd. Na_2SO_4. Upon evaporation of solvent the oily residue (750 mg) was obtained. Usual acetylation of the crude product with Ac_2O (7.5 ml) and pyridine (15 ml), followed by recrystallization from EtOH gave III (426 mg) as colorless needles. mp 199—204°, $[\alpha]_D^{27}$ −136.9° (c=0.71). *Anal.* Calcd. for $C_{23}H_{32}O_5Cl_2$: C, 60.13; H, 7.02. Found: C, 59.94; H, 6.83.

3β-Hydroxyandrost-5-en-16-one Acetate (VIIa)——i) To a solution of III (168 mg) in AcOH (28 ml) containing Ac_2O (3 ml) was added Zn dust (8.5 g) portionwise during 15 min. After boiling under reflux for 13 hr, the reaction mixture was filtered and the precipitate was washed with EtOH. The combined filtrate was concentrated and the residue was extracted with ether. The ether extract was washed with 5% $NaHCO_3$, H_2O and dried over anhyd. Na_2SO_4. After evaporation of solvent, the semicrystalline product was chromatographed on Al_2O_3 (3 g). Elution with *n*-hexane–benzene (8:2 to 6:4) and recrystallization of the eluate from aq. MeOH gave VIIa (41 mg) as colorless leaflets. mp 128—130°, $[\alpha]_D^{18}$ −229.5° (c=0.64). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.44; H, 9.13.

ii) 3β,17β-Dihydroxyandrost-5-en-16-one (VI) (228 mg) was treated with Zn dust (12 g) in AcOH (40 ml) and Ac_2O (4 ml) in the same manner as described in i). The reaction mixture was concentrated and the oily residue obtained was chromatographed on Al_2O_3 (8 g). Elution with *n*-hexane–benzene (10:1 to 6:4) followed by recrystallization from aq. MeOH gave VIIa (140 mg) as colorless leaflets, mp 122—125°. Mixed mp on admixture with the sample obtained in i) showed no depression.

3β-Hydroxyandrost-5-en-16-one (VIIb)——A solution of VIIa (25 mg) in 3% methanolic KOH (4 ml) was refluxed for 1 hr. Recrystallization from aq. MeOH gave VIIb as colorless prisms, mp 167—171°,

15) T. Nambara and M. Katō, *Chem. Ind.* (London), **1967**, 1090.

16) All mp were taken on a micro hot stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solution unless otherwise stated. NMR spectra were determined with JNM 3H-60 spectrometer at 60 Mc in $CDCl_3$ employing $(CH_3)_4Si$ as an internal standard. TLC plate was prepared according to the Stahl's procedure using silica gel H(E. Merck AG) as adsorbent.

$[\alpha]_D^{31}$ −266.2° (c=0.68). *Anal.* Calcd. for $C_{19}H_{28}O_2 \cdot \frac{1}{2}H_2O$: C, 76.72; H, 9.83. Found: C, 76.36; H, 9.77. (Reported mp 168—169°, $[\alpha]_D^{20}$ −255°).[17]

Androst-4-ene-3,16-dione (VIII)——To an ice-cooled solution of VIIb (74 mg) in acetone (10 ml) was added Jones reagent (0.068 ml) and the solution was allowed to stand for 5 min. The reaction mixture was poured into ice-water, extracted with ether, washed with H_2O and dried over anhyd. Na_2SO_4. After evaporation of solvent, the crystalline residue obtained was dissolved in *n*-hexane–benzene (1:1), adsorbed on Al_2O_3 (3.5 g) and recrystallization of the eluate from aq. MeOH gave VIII (28 mg) as colorless prisms. mp 151—153°, $[\alpha]_D^{19}$ −82.5° (c=0.38), UV: λ_{max}^{EtOH} 240 mμ (17,600). *Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.96; H, 9.27. (Reported mp 152—153°, $[\alpha]_D^{19}$ −90.5°).[17]

3β,16β-Dihydroxyandrost-5-en-17-one Diacetate (V)——Prepared from androst-5,16-diene-3β,17-diol diacetate (IV) using the procedure of Mori, *et al.* mp 166—168°, $[\alpha]_D^{27}$ +7.5° (c=0.53). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.85; H, 8.34. (Reported mp 172—173°, $[\alpha]_D^{27}$ +9°).[7]

3β,17β-Dihydroxyandrost-5-en-16-one (VI)——Prepared from 3β,16β-dihydroxyandrost-5-en-17-one diacetate (V) using the procedure of Mori, *et al.* mp 208—211°, $[\alpha]_D^{29}$ −240.0° (c=0.40, MeOH). (Reported mp 207—211°).[7]

3β-Hydroxyandrost-5-en-16-one *p*-Toluenesulfonate (IX)——Prepared from 3β-hydroxyandrost-5-en-16-one (VIIb) using *p*-toluenesulfonyl chloride and pyridine in the usual manner. mp 154—156° (decomp.), $[\alpha]_D^{18}$ −165.6° (c=0.63). *Anal.* Calcd. for $C_{26}H_{34}O_4S$: C, 70.55; H, 7.74. Found: C, 70.50; H, 7.47. (Reported mp 154—155°, $[\alpha]_D^{20}$ −164°).[18]

6β-Hydroxy-3α,5α-cycloandrostan-16-one (X)——A solution of IX (200 mg) and AcOK (260 mg) in 80% aq. acetone (12 ml) was refluxed for 6 hr. After evaporation of solvent the residue was extracted with ether, washed with H_2O and dried over anhyd. Na_2SO_4. The crude product was dissolved in 2% methanolic KOH (4 ml) and refluxed for 1 hr. The mixture was concentrated, extracted with ether, washed with 5% HCl, H_2O and dried over anhyd. Na_2SO_4. The crude product was submitted to the preparative TLC employing silica gel H as adsorbent and benzene–ether (1:1) as developing solvent. Upon exposure to I_2 vapor yellow colored spots appeared immediately in two areas (*Rf* 0.41, 0.48). Elution from the adsorbent corresponding to the lower spot and recrystallization of the eluate from *n*-hexane–acetone gave X (75 mg) as colorless prisms. mp 123—126°, $[\alpha]_D^{30}$ −238.7° (c=0.31). *Anal.* Calcd. for $C_{19}H_{28}O_2$: C, 79.12: H, 9.79. Found: C, 78.87; H, 9.79.

Androsta-3,5-dien-16-one (XI)——A solution of IX (0.3 g) dissolved in DMSO (3 ml) was heated at 80—90° for 4 hr. The reaction mixture was poured into H_2O and extracted with ether. The organic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhyd. Na_2SO_4. After evaporation of solvent the residue obtained was chromatographed on Al_2O_3 (15 g). Elution with *n*-hexane and recrystallization of the eluate from MeOH gave XI (30 mg) as colorless leaflets. mp 110—112°, $[\alpha]_D^{11.5}$ −348.4° (c=3.1). UV: λ_{max}^{MeOH} 228 mμ (18,900), 235 (21,400), 244 (13,800). *Anal.* Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.32; H, 9.60.

Isolation of Zimmermann Complex of 3β-Hydroxyandrost-5-en-16-one——To a solution of VIIb (360 mg) and *m*-dinitrobenzene (210 mg) dissolved in EtOH (20 ml) was added ethanolic 2.5 N KOH (0.8 ml) dropwise under stirring and the reaction mixture was allowed to stand in refrigerator overnight. Upon evaporation of solvent, a dark black solid product was obtained. The crude product was extracted with benzene (100 ml) to remove the unchanged starting material. The filtrate was discarded and the residue was washed again with additional 100 ml of benzene, dissolved in EtOH and passed through acid-washed Al_2O_3 (20 g). The yellow-colored fraction, which showed distinct Zimmermann color promptly upon addition of NaOH solution, was collected and concentrated to dryness *in vacuo* below 25° to provide yellow solid product (212 mg). This crude product was dissolved in *n*-hexane–benzene (1:3) and rechromatographed on acid-washed Al_2O_3 (5 g). Elution with benzene and benzene–ether (4:1) and recrystallization of the eluate from MeOH gave 3β-hydroxy-17ξ-(2,4-dinitrophenyl)androst-5-en-16-one (15 mg) as colorless needles. mp 220—224°, $[\alpha]_D^{21}$ −41.2° (c=0.26). *Anal.* Calcd. for $C_{25}H_{30}O_6N_2$: C, 66.05; H, 6.65; N, 6.16. Found: C, 65.81; H, 6.65; N, 6.24. $\lambda_{max}^{0.05N\ KOH\ in\ EtOH}$ 560 mμ (24,000).

Estimation of Dehydroisoandrosterone Index——The Zimmermann reaction was carried out according to the method of Callow, *et al.*[13] and the absorption spectrum of the colored solution produced was measured by Hitachi Model EPS-2 recording spectrophotometer. The relative extinction value at 520 mμ was expressed as dehydroisoandrosterone index, when the observed value with an equimolar amount of dehydroisoandrosterone was taken as 100.[3]

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17) J. Fajkoš and F. Šorm, *Collection Czechoslov. Chem. Communs.*, **19**, 349 (1954).
18) J. Fajkoš and J. Joska, *Collection Czechoslov. Chem. Communs.*, **26**, 1118 (1961).