Faculty of the Pharmaceutical Sciences of the University of Tokyo and of the Hoshi College of Pharmacy. The author is grateful to them. This work was partly supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which the author is also indebted.

Summary

It was found that itaconitin, a yellow coloring matter of a mould *Aspergillus itaconicus* KINOSHITA, possesses a 3,5-dienoic acid system which is converted into an aromatic ring by the action of acetic anhydride to give acetylanhydroitaconitin. Oxidation of hexahydroitaconitin with potassium permanganate, as well as the ozonolysis of the same substance yielded 2-methylnonanedioic acid. From these and other experimental data including nuclear magnetic resonance spectra, the structural formula of itaconitin and all its derivatives hitherto prepared were conclusively established.

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14. Toshio Nambara and Motohiko Katō : Analytical Chemical Studies on Steroids. IV*1. The Zimmermann Reaction of 16- and 17-Oxosteroids.*2

(*Faculty of Pharmaceutical Sciences, University of Tokyo**3)

The Zimmermann reaction has been widely used in the field of biochemistry as well as organic chemistry for the detection and determination of the compounds having active methylene group, particularly, of the oxosteroids. Since the reaction was discovered by Zimmermann[4] in 1935, several workers have investigated its specificity and applicability.[5~8] With regards to the reaction mechanism, it has been accepted that *m*-dinitrobenzene condenses with the active methylene group to yield a Meisenheimer-type compound (IIa)[9~12] as shown in Chart 1. This mechanism had not apparently been

*1 The previous papers, "Colorimetric determination of cholestan-3α-ol in the presence of cholestan-3β-ol and cholesterol,"[1] "Reactions of the 16-double bond of 5α,14β-androst-16-ene,"[2] and "Chemistry of 3β,12-dihydroxy-5α-androstan-17-ones"[3] were entitled as Part I, II and III of this series, respectively.

*2 A part of this paper was presented at the 84th Annual Meeting of Pharmaceutical Society of Japan in Tokyo (April, 1964).

*3 Motofuji-cho, Bunkyo-ku, Tokyo (南原利夫, 加藤元彦).

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

No.	Compounds	Concentration $\mu M/0.2$ ml.	Absorbance at (mμ) 400	440	480	520	600	Dehydroisoandrosterone index
0	3β-Hydroxyandrost-5-en-17-one (Dehydroisoandrosterone)[s]	.3564	.178	.256	.422	.533	.278	100
1	3α-Hydroxy-5α-androstan-17-one[s]	.3313	.146	.206	.375	.493	.260	99
2	3β-Hydroxy-5α-androst-14-en-17-one acetate[a]	.3808	.047	.020	.016	.010	.000	2
3	3β-Hydroxyandrosta-5,14-dien-17-one acetate[b]	.3099	.134	.112	.094	.051	.009	11
4	3β,14α-Dihydroxy-5α-androstan-17-one 3-acetate[b]	.3808	.002	.017	.028	.030	.013	5
5	3β,14α-Dihydroxyandrost-5-en-17-one 3-acetate[b]	.2677	.000	.005	.012	.013	.009	3
6	14α-Hydroxyandrost-4-ene-3,17-dione[c]	.8382	.337	.288	.297	.278	.232	22
7	3β,16β-Dihydroxy-5α-androstan-17-one	.1460	.044	.044	.037	.035	.024	16
8	3β,16β-Dihydroxy-5α-androstan-17-one dimesylate	.2053	.022	.047	.064	.054	.018	18
9	16β-Hydroxyandrost-4-ene-3,17-dione acetate[d]	.7203	.244	.208	.199	.177	.161	16
10	3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one[e]	.3075	.002	.011	.029	.026	.014	6
11	3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one diacetate[e]	.2856	.008	.021	.029	.019	.008	4
12	3,16β-Dihydroxyestra-1,3,5(10)-trien-17-one diacetate[e]	.3588	.025	.030	.034	.027	.015	5
13	3β-Hydroxy-16α-bromo-5α-androstan-17-one acetate[f]	.2754	.008	.244	.338	.494	.244	120
14	3β-Hydroxy-16β-bromo-5α-androstan-17-one acetate[f]	.2934	.045	.142	.413	.616	.291	140
15	3β-Hydroxy-16α-bromoandrost-5-en-17-one[f]	.2344	.019	.083	.280	.464	.209	132
16	3-Hydroxy-16α-bromoestra-1,3,5(10)-trien-17-one acetate[g]	.3060	.038	.114	.316	.486	.257	106
17	3-Hydroxy-16β-bromoestra-1,3,5(10)-trien-17-one[g]	.3504	.045	.154	.454	.684	.359	130
18	3-Methoxy-16β-methylestra-1,3,5(10)-trien-17-one[h]	.4105	.068	.102	.128	.100	.042	16
19	3β,12α-Dihydroxy-5α-androstan-17-one[i]	.3269	.134	.207	.267	.336	.135	69
20	3β,12β-Dihydroxy-5α-androstan-17-one[i]	.3074	.163	.275	.447	.495	.180	108
21	3β,12α-Dihydroxy-5α-androstan-17-one diacetate[i]	.2740	.087	.135	.210	.267	.101	65
22	11β-Hydroxyandrost-4-ene-3,17-dione[j]	.2943	.357	.322	.416	.481	.287	109
23	11β-Hydroxyandrost-1,4-diene-3,17-dione[k]	.4244	.209	.279	.500	.627	.281	99
24	Androst-4-ene-3,11,17-trione[s]	.3315	.375	.362	.455	.539	.347	109
25	6β-Hydroxy-3,5-cycloandrostan-17-one[l]	.3314	.200	.254	.440	.587	.289	118
26	3α-Hydroxy-19-nor-5α-androstan-17-one[m]	.3143	.134	.196	.352	.467	.220	99
27	19-Hydroxyandrost-4-ene-3,17-dione[n]	.3866	.390	.375	.437	.499	.338	86
28	3β-Hydroxy-5α-androstan-16-one[f]	.4107	.133	.203	.367	.495	.227	81
29	3-Hydroxyestra-1,3,5(10)-trien-16-one acetate[o]	.3557	.101	.092	.099	.108	.077	20
30	3β,17β-Dihydroxyandrost-5-en-16-one[p]	.3196	.008	.021	.029	.020	.012	4
31	3β,17β-Dihydroxyandrost-5-en-16-one diacetate[p]	.2597	.000	.010	.016	.010	.006	3
32	3,17β-Dihydroxyestra-1,3,5(10)-trien-16-one[q]	.4145	.051	.087	.108	.081	.034	13
33	3,17β-Dihydroxyestra-1,3,5(10)-trien-16-one diacetate[q]	.2889	.008	.018	.020	.014	.008	3
34	3β-Hydroxy-17β-bromo-5α-androstan-16-one acetate[q]	.2825	.070	.073	.085	.086	.056	20
35	3β-Hydroxy-5α,14β-androstan-17-one acetate[b]	.2916	.103	.208	.363	.478	.227	110
36	3β-Hydroxy-16β-bromo-5α,14β-androstan-17-one acetate[r]	.3185	.079	.148	.368	.562	.365	118
37	3β,17α-Dihydroxy-5α,14β-androstan-16-one diacetate[r]	.2561	.043	.034	.027	.019	.015	5
38	3β-Hydroxy-5α,14β-androstan-16-one acetate[r]	.2571	.138	.164	.251	.302	.185	79

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c) D.H. Peterson, S.H. Eppstein, P.D. Meister, H.C. Murray, H.M. Leigh, A. Weintraub, L.M. Reinecke: *Ibid.*, 75, 5768 (1953).
d) S. Noguchi: Yakugaku Zasshi, 81, 385 (1961). e) Reference 27.
f) J. Fajkoš: Collection Czechoslov. Chem. Communs., 20, 312 (1955); 24, 766 (1959).
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q) Reference 20. r) Reference 26. s) Commercially available.

criticized until 1960, when Neunhoeffer, *et al.*[13] pointed out that oxidation step is involved in this reaction and the excess of *m*-dinitrobenzene plays a role of oxidizing agent. The similar explanations have further been advanced by other groups independently.[14,15] The authors have much interest in this reaction since it is of great use for the biochemical investigations on steroids. The present paper deals with the scope and mechanism of the Zimmermann reaction as to some 16- and 17-oxosteroids, in particular, 16-halo-17-oxo derivatives, and the stereochemical interpretation for the influences of environment around oxo group on this reaction.

The initial project was directed to the comparative studies on the rate of coloration produced from various steroids with ketone in ring D. The absorption spectrum of the colored solution obtained by the method of Callow, *et al.*[16] was measured and the extinction value at 520 mμ was compared with that of dehydroisoandrosterone taken as standard. The results observed are summarized in Table I.

Almost all α-substituted 17-oxosteroids, namely, 16-hydroxy, acetoxy, mesyloxy and methyl derivatives (No. 7, 10, 9, 11, 12, 8, 18) gave negative results, whereas 16-bromo-17-oxosteroids (No. 13, 14, 15, 16, 17) showed an exceptional behaviour providing the typical Zimmermann color. Moreover, 16-halo-17-oxosteroids produced coloration much more rapidly than the parent 17-oxosteroids. With the former purple color appeared promptly just after addition of reagents and reached to the maximum intensity in a few minutes, while with 16-unsubstituted 17-oxosteroids extinction value still increased even after 30 minutes as illustrated in Fig. 1. Furthermore, there could be seen no significant difference in the rate of color development between the two epimeric 16-bromo derivatives. Roy[8] also observed this interesting behaviour of 16-halo-17-oxosteroids and tried to clarify the mechanism, but it was unsuccessful to isolate the Zimmermann complex[17,18] in a crystalline state and in consequence, confirm the mechanism conclusively. Hence, the attempt was made to separate the Zimmermann complex from the colored solution produced when dehydroisoandrosterone (Ia) and its 16α-bromo derivative (Ib) were used respectively. The Zimmermann reaction was carried out on the preparative scale and the reaction mixture was chromatographed on acid-washed alumina followed by recrystallization, hereupon 3β-hydroxy-16ξ-(2,4-dinitrophenyl)androst-5-en-17-one (IVa), m.p. 186~188°, could be successfully isolated as colorless needles. Usual acetylation of IVa with acetic anhydride and pyridine provided 3-acetate (IVb), m.p. 200~202°, quantitatively. Both Zimmermann complexes thus obtained from dehydroisoandrosterone and its 16α-bromo derivative proved to be entirely identical in all respects by mixed melting point measurement and

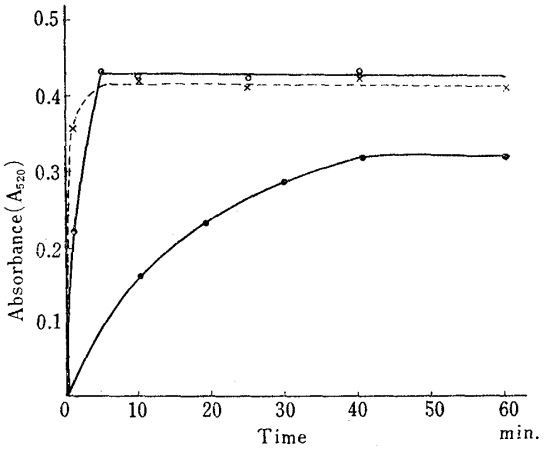

Fig. 1. The Rate of the Zimmermann Reaction with 0.2 μ*M* of Dehydroisoandrosterone (—•—), 3β-Hydroxy-16α-bromoandrostan-17-one Acetate (---x---) and 3β-Hydroxy-16β-bromoandrostan-17-one Acetate (—o—)

13) O. Neunhoeffer, K. Thewalt, W. Zimmermann : Z. physiol. Chem., Hoppe-Seyler's, **323**, 116 (1961). Also see M. Ishidate, T. Sakaguchi : Yakugaku Zasshi, **70**, 439 (1950).
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16) N. H. Callow, R. K. Callow, C. W. Emmens : Biochem. J., **32**, 1312 (1938).
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infrared spectra comparison (Fig. 2). With respect to the structure of the Zimmermann complex, configuration of dinitrophenyl group at C-16 could not be elucidated, but their nuclear magnetic resonance spectra (Fig. 3) were indicative of 16α rather than 16β on the basis of the following assumption. An examination of Dreiding models shows that 2,4-dinitrophenyl group being introduced at 16β, the steric interaction with 18-methyl group might prevent the free rotation of C-16-substituent around C-16-ϕ bond and accordingly, steric requirement that benzene ring is to be located above C-13-angular group might be dominant to affect the chemical shift of 18-methyl protons by ring current effect.[19] However, in fact, comparing the chemical shifts of angular methyl groups of Na with those of dehydroisoandrosterone, any upfield shift by shielding effect of C-16-aryl group was not observed. Therefore, the orientation of 2,4-dinitrophenyl group might be assigned to α rather than β. It is well-known that protonation at C-16 in C/D-*trans* steroid would take place from the less hindered α-side to yield 16β-substituent, but in this case the significant interaction between bulky dinitrophenyl and 18-methyl group would favor to furnish the more thermodynamically stable 16α-substituent.

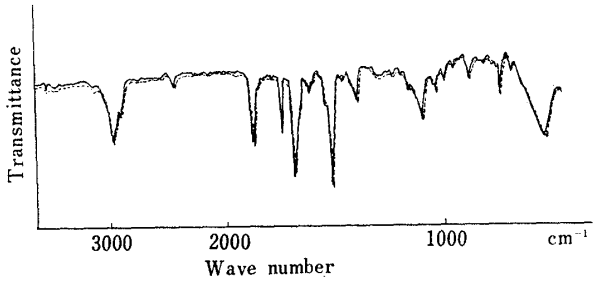

Fig. 2. The Infrared Spectra of the Zimmermann Complexes obtained from Dehydroisoandrosterone (-----) and 16α-Bromodehydroisoandrosterone (——)

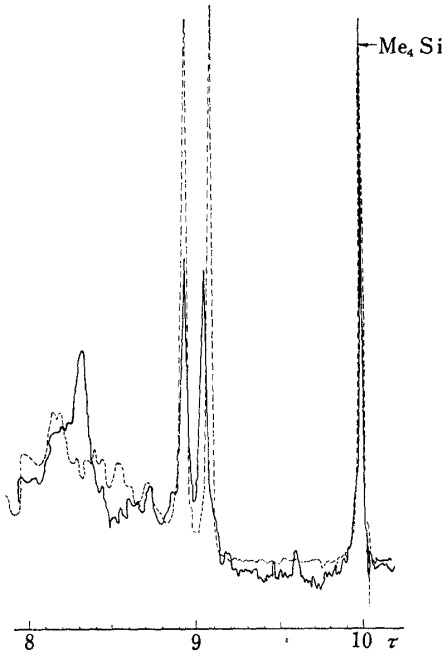

Fig. 3. The Nuclear Magnetic Resonance Spectra of Dehydroisoandrosterone (------) and its Zimmermann Complex (——) (Varian A-60; 60 Mc.; $CDCl_3$ Solution)

Recently, Neunhoeffer, *et al.*[13] and King, *et al.*[14] have reported the isolation of 3,3′-dinitroazobenzene and *m*-nitroaniline from the reaction mixture respectively, which were regarded as the evidence of oxidation step involved in the Zimmermann reaction. In order to obtain exact information as to the mechanism, examinations were made on characterizing the reaction products derived from *m*-dinitrobenzene. A method for identification and approximate estimation of the reduction product was established by means of thin-layer chromatography with use of Silica gel G as adsorbent, and stannous chloride and 4-dimethylaminocinnamaldehyde as staining reagents. As can be realized by chromatogram illustrated in Fig. 4, characterization of possible products was satisfactorily attained. In the case of dehydroisoandrosterone, *m*-nitroaniline was detected as a major product accompanied with some other minor spots, while in the case of its 16α-bromo derivative, any distinct difference from that of blank test was not recognized. It has already been postulated that the Zimmermann reaction of 17-oxosteroid would proceed through the initial alkali-catalyzed formation of carbanion at C-16 and the nucleophilic

19) L. M. Jackman : "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 125 (1959), Pergamon Press, New York.

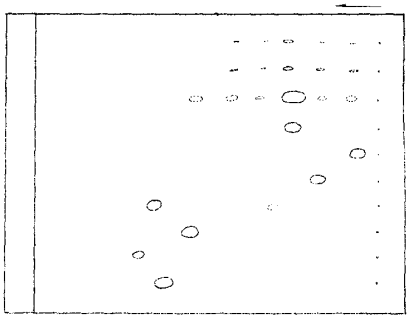

Fig. 4. Thin-layer Chromatogram of Reduction Products of *m*-Dinitrobenzene by the Zimmermann Reaction using Dehydroisoandrosterone and its 16α-Bromo Derivative

Adsorbent: Silica gel G (E. Merck Co.), Layer thickness 0.3 mm.
Developing solvent: benzene
Developing time: 25 min.(at room temperature)
Detection: 0.25% $SnCl_2$ in 1% HCl and 0.5% 4-dimethylaminocinnamaldehyde in EtOH

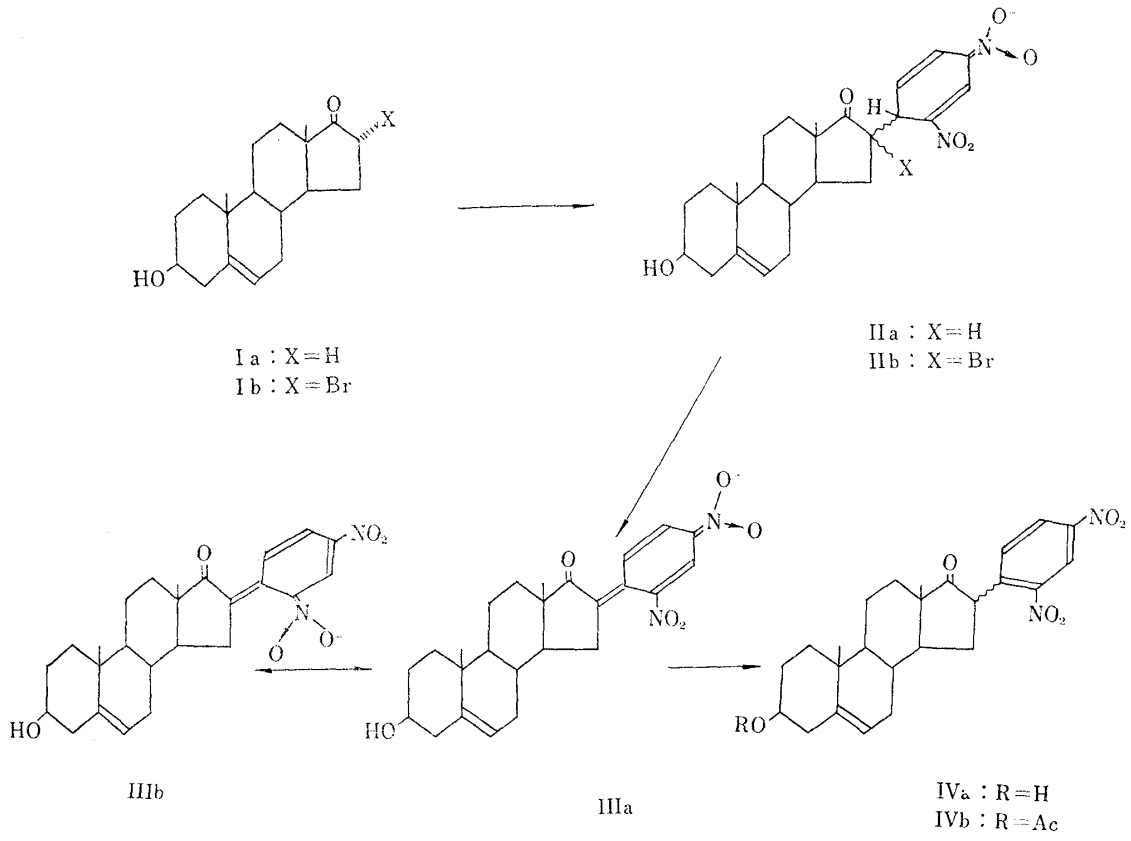

Chart 1.

attack of the resultant anion on the activated position of *m*-dinitrobenzene. For the formation of the Zimmermann chromophore (III) from IIa negative charge derived from carbanion must be replaced to hydrogen atom and then, bonding electron of the latter must also be displaced. This mechanism requires the presence of an oxidizing agent, whose function would presumably be performed by aromatic nitro compounds. The thin-layer chromatogram rationalized the participation of *m*-dinitrobenzene in the oxidation step when dehydroisoandrosterone was used for the reaction. On the other hand, its 16α-bromo derivative being employed, the result indicated that *m*-dinitrobenzene would not be concerned in this oxidation but bromo cation produced during the

course would take its place. However, it is not obvious whether bromo cation would be liberated in the initial step or in the subsequent one, in other words, whether the reaction would proceed by way of IIa or IIb as an intermediate.

It is also of particular interest that in comparison with 17-oxosteroid, androstan-16-one (No. 28, 36) showed somewhat weak reaction and estrone-16 (No. 29) gave almost negative result. This finding seems to be quite surprising since the ketone at C-16 has two active methylene groups available in contrast to 17-ketone possessing only one α-methylene, but it is fairly in good accordance with that on the related reactions of these oxosteroids. Fishman[20] reported that enol acetate formation was readily accomplished with androstan-17-one, whereas under the same conditions the reaction is somewhat difficult with androstan-16-one, and actually impossible with estrone-16. The similar situation was also observed on the relative ease of bromine uptake. On the other hand it is generally accepted that the rate-determining step in these reactions is deprotonation of α-methylene group, that is, enolization of ketone. A possible explanation for the difference in enolization can be advanced on the basis of recent works[21~24] on the conformation of cyclopentanones and in particular, the fused cyclopentane that constitutes ring D of steroids. Of two possible conformations ring D with a ketone at C-17 very likely exists in half-envelope form (Cs), while with a ketone at C-16 the half-chair form (C2). Enolization of 17-ketone requires no conformational change, whereas that of the C-16-ketone would require a change from the more favored C2 to the Cs conformation. Thus, the difference in the Zimmermann reaction described above can be advantageously explained by the assumption that the rate-determining step in this reaction would be deprotonation of α-methylene as well, relative ease of which depends on the conformation of ring D. Nevertheless no plausible interpretation for significant difference between androstan-16-one and estrone-16 is now available. It may be ascribed to the long-range conformational effects of aromatic A-ring.[25]

All 17-substituted 16-oxosteroids (No. 30, 31, 32, 33, 34) so far examined gave no typical coloration. Based on the previous observations that enolization of 16-ketone is directed predominantly toward C-17 rather than C-15, it is possible to account for the inertness of 17-hydroxy or acetoxy derivatives but difficult to explain the different behaviour of 17-bromo compound (No. 34) from that of 16-bromo-17-ketones.

The 17-oxosteroids having modified ring A or C-10-angular group (No. 25, 26, 27) showed the typical Zimmermann color as usual. Of the oxygen substituents in ring C, 11β-, 12β-hydroxyl and 11-oxo groups (No. 22, 23, 20, 24) did not substantially affect, while 12α-acetoxyl and hydroxyl groups (No. 21, 19) slightly decreased the rate of color development. The compounds having 14α-hydroxyl group or double bond in ring D gave no characteristic coloration. The hydroxyl group at 14α (No. 4, 5, 6) may exert a steric and/or electronic effects to a certain extent on the reactivities of 17-ketone or adjacent methylene group. The Δ^{14}-compounds (No. 2, 3) showed purple color promptly, which changed immediately to the uncharacteristic one, and finally gave apparently negative result. With the latter compounds 16-methylene group activated by both adjacent 17-ketone and double bond would accept condensation more readily to afford the Zimmermann chromophore, which in turn may be subjected to decomposition with ease probably because of its unstable conjugation system.

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25) D. H. R. Barton, *et al.* : *Ibid.*, **1956**, 932; **1957**, 935; **1960**, 1297.

The unusual ring fusion of C/D juncture did not substantially influence the coloration and 14β-steroids showed similar behaviours as the corresponding C/D-*trans* steroids. The difference in the Zimmermann reaction between 16- and 17-oxosteroids (No. 38, 35) was likewise consistent with the recent observations on the stereochemistry of ring D in 14β-series.[26] In addition, 16β-bromo-17-oxosteroid (No. 36) furnished the Zimmermann chromophore promptly, and 17-substituted 16-oxo compound (No. 37) showed almost negative result.

Further studies on the Zimmermann reaction of other oxosteroids in connection with stereochemistry are being conducted in this laboratory.

Experimental*4

Samples——Almost all samples, not mentioned below, were either prepared by the authors following the known methods or gifted from other laboratories, which are acknowledged elsewhere.

3β,16α-Dihydroxy-5α-androstan-17-one (No. 7)——A solution of 5α-androst-16-ene-3β,17-diol diacetate (700 mg.) in $CHCl_3$ (45 ml.) containing perbenzoic acid (0.0028*M*) was allowed to stand for 20 hr. at room temperature. The solution was diluted with Et_2O and washed with *N* NaOH and then H_2O. After drying the organic layer and evaporation of solvent an oily residue (400 mg.) was obtained. To the solution of the crude product (270 mg.) in MeOH (32 ml.) was added 6*N* H_2SO_4 (8 ml.), and the mixture was allowed to stand at room temperature for 5 days. After dilution with AcOEt and washing with cold *N* NaOH and H_2O, the organic layer was dried and evaporated to give semisolid residue (220 mg.). The product was dissolved in hexane-benzene (1:1) and chromatographed on Al_2O_3. Elution with $CHCl_3$-MeOH (9:1) yielded a crystalline product (115 mg.), which was recrystallized from Me_2CO-hexane to give colorless plates. m.p. 184~187°, $[\alpha]_D^{18.5}$ +97° (c=0.61). *Anal.* Calcd. for $C_{19}H_{30}O_3 \cdot H_2O$: C, 70.33; H, 9.94. Found: C, 69.96; H, 9.60. Usual acetylation with Ac_2O and pyridine gave 3, 16-diacetate,[27] m.p. 184~185°.

3β,16α-Dihydroxy-5α-androstan-17-one 3,16-Dimesylate (No. 8)——To a solution of 3β,16α-dihydroxy-5α-androstan-17-one (88 mg.) dissolved in pyridine (0.5 ml.) was added methanesulfonyl chloride (0.1 ml.) dropwise under cooling and the mixture was allowed to stand in refrigerator overnight. The reaction mixture was poured into ice-water and the precipitated product was collected, washed with H_2O and recrystallized from MeOH to give 3,16-dimesylate as colorless needles. Yield, 101 mg. m.p. 163.5~164°, $[\alpha]_D^{18.5}$ +77° (c=1.04). *Anal.* Calcd. for $C_{21}H_{34}O_7S_2$: C, 54.52; H, 7.41. Found: C, 54.60; H, 7.21.

Estimation of Dehydroisoandrosterone Index——The Zimmermann reaction was carried out according to the procedure of Callow, *et al.*,[16] and the absorption spectrum of the colored solution produced was measured by Cary Model 11 recording spectrophotometer. The relative extinction value at 520 mμ was represented as dehydroisoandrosterone index, when the observed value with an equimolar amount of dehydroisoandrosterone was taken as 100.

Procedure: Into a glass-stoppered brown test-tube were measured test solution (0.2 ml.) in EtOH or EtOH (0.2 ml.) for blank, 2% ethanolic solution (0.2 ml.) of *m*-dinitrobenzene and ethanolic 2.5*N* KOH solution (0.2 ml.). The tubes were then stoppered, gently shaken, and placed in a thermostat at 25±1° for 60 min. At the end of 60 min. the reaction mixture was diluted to 10 ml. with EtOH and the contents mixed. The colored solution obtained was submitted to spectral measurement with use of the blank test.

Measurement of Reaction Velocity with 16-Bromo-17-oxosteroids——The Zimmermann reaction was carried out in the same manner as described above with use of 0.2 μM each of dehydroisoandrosterone and two epimeric 3β-acetoxy-16-bromo-5α-androstan-17-ones. The reaction mixtures were incubated for 1, 5, 10, 25, 40 and 60 min., respectively and the absorption spectra were measured by Hitachi Model EPS-2U recording spectrophotometer with scanning time of 1 min. Extinction values at 520 mμ observed were plotted against the incubation time.

Isolation of Zimmermann Complex

i) From 3β-Hydroxyandrost-5-en-17-one (dehydroisoandrosterone)——To a solution of dehydroisoandrosterone (2 g.) and *m*-dinitrobenzene (1.2 g.) dissolved in EtOH was added ethanolic 2.5*N* KOH (4.3 ml.) dropwise and the whole volume was brought to 120 ml. by addition of EtOH. The 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 (300 ml.) to remove the unchanged starting material.

*4 All melting points are uncorrected and all rotations were measured in $CHCl_3$. The NMR spectra were measured by a Varian A-60 Spectrometer at 60 Mc.p.s., in $CDCl_3$ with use of Me_4Si as an internal standard. The IR spectra were measured in $CHCl_3$ by Kōken Model DS-301 Spectrophotometer.

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The filtrate was discarded, and the residue was washed again with additional 200 ml. of benzene, dissolved in EtOH and passed through acid-washed Al_2O_3 (70 g.). A 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 (1.2 g.). A solution of the crude product dissolved in hexane-benzene (1:4) was rechromatographed on acid-washed Al_2O_3 (30 g.). Elution with benzene and benzene-Et_2O (4:1) afforded a pale yellow crystalline product. Recrystallization from hexane-$CHCl_3$ gave 3β-hydroxy-16ξ-(2,4-dinitrophenyl)androst-5-en-17-one (Ⅳa) as colorless needles. Yield, 338 mg. m.p. 186~188°, $[\alpha]_D^{19.8}$ −161° (c=0.60). *Anal.* Calcd. for $C_{25}H_{30}O_6N_2$: C, 66.05; H, 6.65; N, 6.16. Found: C, 66.10; H, 6.93; N, 6.06. A solution of Ⅳa dissolved in ethanolic 0.05*N* KOH showed purple coloration having an absorption maximum at 520 mμ (ε 33,000). Corker, *et al.*[28] isolated the same compound by acidifying the reaction mixture obtained from dehydroisoandrosterone, *m*-dinitrobenzene and tetraethylammonium hydroxide, and reported it m.p. 186~187°, $[\alpha]_D$ −170°.

3-Acetate (Ⅳb): Ⅳa (156 mg.) was dissolved in pyridine (1.5 ml.) and Ac_2O (0.75 ml.) and the solution was allowed to stand at room temperature overnight. On usual work-up and recrystallization from EtOH 3-acetate was obtained as colorless needles. Yield, 150 mg. m.p. 200~202°, $[\alpha]_D^{19.8}$ −41° (c=0.56). *Anal.* Calcd. for $C_{27}H_{32}O_7N_2$: C, 65.31; H, 6.50; N, 5.64. Found: C, 65.19; H, 6.50; N, 5.00.

ii) **From 3β-Hydroxy-16α-bromoandrost-5-en-17-one**——With use of 3β-hydroxy-16α-bromoandrost-5-en-17-one (200 mg.) the Zimmermann reaction was carried out in the same manner as described above. On work-up in the above way, Zimmermann complex was obtained as colorless needles. Yield, 25 mg. m.p. 186~188°, $[\alpha]_D^{18.5}$ −160° (c=0.61). The mixed melting point on admixture with the sample described in i) showed no depression and the IR spectra of two samples were identical in every respect.

Characterization of the Reduction Products of *m*-Dinitrobenzene——Of several possible reduced compounds of *m*-dinitrobenzene, *m*-nitronitrosobenzene,[29] N-(*m*-nitrophenyl)hydroxylamine,[30] 3,3′-dinitroazobenzene,[31] 3,3′-dinitroazoxybenzene[30] and 3,3′-dinitrohydrazobenzene[32] were synthesized by the published procedures. TLC plate was prepared and activated according to the Stahl's procedure with use of Silica gel G (E. Merck Co.) as adsorbent. Detection of the spots was readily accomplished by pink coloration upon spraying of 0.25% $SnCl_2$ solution in 1% HCl and then 0.5% ethanolic solution of 4-dimethylaminocinnamaldehyde. The coloration was also observed without spraying of $SnCl_2$ solution prior to the use of 4-dimethylaminocinnamaldehyde solution. Approximate estimation of each spot was effected by comparison of intensity of the stain on chromatogram. Authentic samples of the possible reduction products were run parallel to the test samples using benzene as developing solvent. To a solution of dehydroisoandrosterone (100 mg.) and *m*-dinitrobenzene (60 mg.) dissolved in EtOH (6 ml.) was added ethanolic 2.5*N* KOH solution (3 ml.), and the mixed solution was incubated at 25±1° for 60 min. After treatment of the reaction mixture in the same manner as described above, the solid residue was extracted with benzene and filtered. The filtrate was concentrated *in vacuo* to dryness and the residue hereby obtained was dissolved in $CHCl_3$, submitted to TLC. Without previous reduction step, a main spot was detected at Rf value 0.28 corresponding to that of *m*-nitroaniline. On previous treatment with $SnCl_2$ solution additional a couple of minor spots (Rf 0.45, 0.56) were detected but could not be characterized. The adsorbent corresponding to the main spot was collected and eluted with Et_2O. Upon evaporation of solvent a yellow crystalline product (0.8 mg.), m.p. 108~111°, was obtained and proved to be identical with the authentic sample of *m*-nitroaniline by mixed melting point measurement, IR spectral and gas chromatographic comparison. On the other hand the reaction was carried out in the same manner using an equimolar amount of 16α-bromodehydroisoandrosterone and the benzene extract was submitted to TLC.

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Summary

The specificity of the Zimmermann reaction was examined with 38 kinds of 16- and

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17-oxosteroids. The influences of environmental situation around oxo group on the reactivities were observed and interpreted in terms of stereochemistry. Of 16-substituted 17-oxosteroids only 16-monohalo derivative showed an exceptional behaviour giving a typical Zimmermann color. By treatment of the colored solution produced from 16α-bromodehydroisoandrosterone, the Zimmermann complex, 3β-hydroxy-16ξ-(2,4-dinitrophenyl)androst-5-en-17-one, m.p. 186~188°, was obtained and proved to be identical with that of dehydroisoandrosterone.

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