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Analytical Chemical Studies on Steroids. XX. Zimmermann Reaction and Deuterium Exchange Reactivity of Oxosteroids and Related Compounds[1]

Motohiko Katō,[2a] Masako Ohnishi[2b] and Toshio Nambara[2c]

Faculty of Pharmaceutical Sciences, University of Tokyo,[2a] National Cancer Center Research Institute[2b] and Pharmaceutical Institute, Tohoku University School of Medicine[2c]

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The relationship between Zimmermann reaction and base-catalyzed deuterium exchange reactivity of 16- and 17-oxosteroids together with some simple ketones has been investigated. With regard to the simple ketones such as cyclopentanone (I), cyclohexanone (II) and diethyl ketone (III) the sequential order of the deuterium exchange activity was found to be in qualitative agreement with that of the rate of Zimmermann reaction as follows: I>II≈III. The Zimmermann complex derived from cholestan-3-one, mp 178—180°, was isolated and assigned to the structure of 2α-(2,4-dinitrophenyl)cholestan-3-one on the basis of the nuclear magnetic resonance spectrum. The spectroscopic properties of Zimmermann complexes produced from the cyclic ketones are also described.

In the previous papers of this series the specificity of Zimmermann reaction was investigated with various kinds of 16-and 17-oxosteroids, and the reaction mechanism was clarified with these steroidal ring D ketones.[3] The initial step of Zimmermann reaction was supposed to be deprotonation of α-methylene adjacent to oxo group, that is, the formation of carbanion in basic medium. This reaction appeared to have common rate-determining step to H–D exchange of the ketone as racemization and halogenation. Therefore the authors have attempted to examine the relationship between Zimmermann reaction and deuterium exchange reactivity of 16- and 17- oxosteroids together with some simple ketones.

The first project was directed to the inspection of the spectroscopic properties of Zimmermann complexes derived from diethyl ketone, cyclopentanone, cyclohexanone and cholestan-3-one. The desired compounds were obtained through either the reaction of the pyrrolidyl enamine with 2,4-dinitrochlorobenzene[4] or Zimmermann reaction followed by chromatographic purification on acid-washed alumina. The structure of Zimmermann complex derived from cholestan-3-one was characterized to be 2α-(2,4-dinitrophenyl)cholestan-3-one based upon the following evidence of nuclear magnetic resonance (NMR) spectrum (see Fig. 1). Three groups of peaks in the range of 1 to 3τ can be assigned to three protons on the benzene ring, namely Ha (1.20τ), Hb (1.60τ) and Hc (2.44τ) where Hb is split into a quartet by coupling with Ha (J=3 cps) and Hc (J=9 cps). The signal due to the CH group attached to the dinitrobenzene residue appears at 5.59 τ as a quartet (J_{AX}=6 cps, J_{BX}=13 cps), whose pattern coincides with that of 2α-acetoxycholestan-3-one of four possible α-ketol acetates.[5]

1) Part XIX: *J. Chromatog.*, 34, 526 (1968).
2) Location: *a*) *Hongo-7-chome, Bunkyo-ku, Tokyo*; *b*) *Tsukiji, Chuo-ku, Tokyo*; *c*) *Kita-4-bancho, Sendai*. To whom any inquiries should be addressed.
3) *a*) T. Nambara and M. Katō, *Chem. Pharm. Bull.* (Tokyo), **13**, 78 (1965); *b*) *Idem, ibid.*, **13**, 1435 (1965); *c*) T. Nambara, M. Katō, R. Imanari and T. Kudo, *ibid.*, **16**, 126 (1968); *d*) T. Nambara and M. Katō, *Chem. Ind.* (London), **1967**, 1703.
4) *a*) M.E. Kuehne, *J. Am. Chem. Soc.*, **81**, 5400 (1959); *b*) *Idem, ibid.*, **84**, 837 (1962).
5) K.L. Williamson and W.S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961).

It is noteworthy that the orientation of displacement of the Zimmermann chromophore is in accordance with the direction of enolization in spite of having two α-methylene groups. This finding is consistent with the result of Zimmermann reaction of 16-oxosteroid, which similarly possesses active methylenes on both sides of the oxo group.[3c]

The spectroscopic constants of these Zimmermann complexes in ethanolic alkaline medium were listed in Table I. It is of interest that Zimmermann chromophore derived from the six-membered ring ketones shows the diminished absorption intensity as compared with that of the five-membered ketones. These properties of Zimmermann complexes in *aci*-form may be interpreted in terms of Turner-Voitle's explanation, which involves the spectroscopic properties of the cyclic ketones bearing exomethylene at α-position.[6]

As a model experiment the relationship between deuterium exchange reactivity and Zimmermann reaction

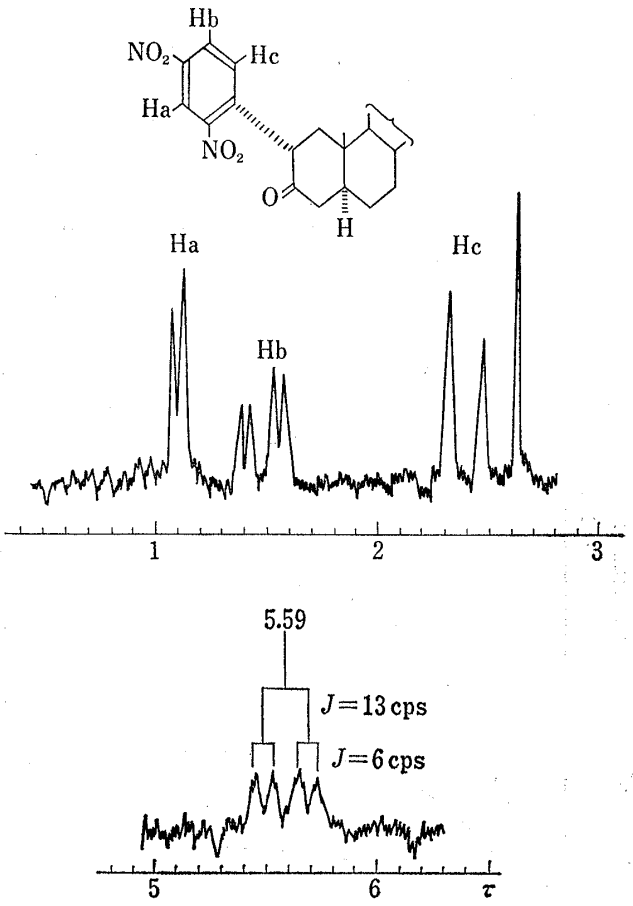


Fig. 1. The NMR Spctrum of the Zimmermann Complex Derived from Cholestan-3-one

varian A-60; 60 Mc; $CDCl_3$ solution

Table I. The Spectroscopic Properties of the Zimmermann Complexes Derived from Oxosteroids and Related Compounds in Ethanolic Alkaline Medium[a]

Compounds	λ_{max} (mμ)	ε_{max}
2-(2,4-Dinitrophenyl)cyclopentanone	535	24000
2-(2,4-Dinitrophenyl)cyclohexanone	560	12000
2-(2,4-Dinitrophenyl)pentan-3-one	540	4700
3β-Hydroxy-16ξ-(2,4-dinitrophenyl)androst-5-en-17-one	520	33000
3β-Hydroxy-17ξ-(2,4-dinitrophehyl)androst-5-en-16-one	560	24000
2α-(2,4-Dinitrophenyl)cholestan-3-one	564	13000

a) ethanolic 0.05 N KOH solution

was examined with the simple ketones such as cyclopentanone, cyclohexanone and diethyl ketone. Several methods have already been proposed for determination of the deuterium exchanged.[7] Of these techniques the nuclear magnetic resonance spectrometric method,

6) R.B. Turner and D.M. Voitle, *J. Am. Chem. Soc.*, **73**, 1403 (1951).

7) R.N. Jones, A.R.H. Cole and B. Nolin, *J. Am. Chem. Soc.*, **74**, 5662 (1952); W. von E. Doering, M.R. Willcott, III and M. Jones, Jr., *ibid.*, **84**, 1224 (1962); H.L. Goering, D.L. Towns and B. Dittmar, *J. Org. Chem.*, **27**, 736 (1962); N. Tamiya, *Bull. Chem. Soc. Japan.*, **35**, 863 (1962); A.T. Bottini and A.J. Davidson, *J. Org. Chem.*, **30**, 3302 (1965).

established by Kawazoe, *et al.*,[8] seemed to be more suitable for a series of determination of exchange rate with respect to feasibility in preparation of the test sample and in quantitative measurement. The ketones to be examined were dissolved in deuterium oxide–deuteriomethanol containing potassium carbonate and also *tert*-butanol as an internal reference. In order to overcome the difficulties due to pH alteration of the resulting solution and overlapping of the α-methylene signals, two sets of combination, namely diethyl ketone–cyclopentanone and diethyl ketone–cyclohexanone, were chosen for measurement of the spectra. The nuclear magnetic resonance spectra of the sample solutions thus prepared were taken and the areal intensities of α-methylene peaks referring to that of the internal standard were determined. The number of hydrogens exchanged with deuterium was estimated along with the reaction time. As shown in Table II the semiquantitative results tell us that the order of reactivity of deuterium exchange is obviously as follows: cyclopentanone>cyclohexanone≈diethyl ketone.

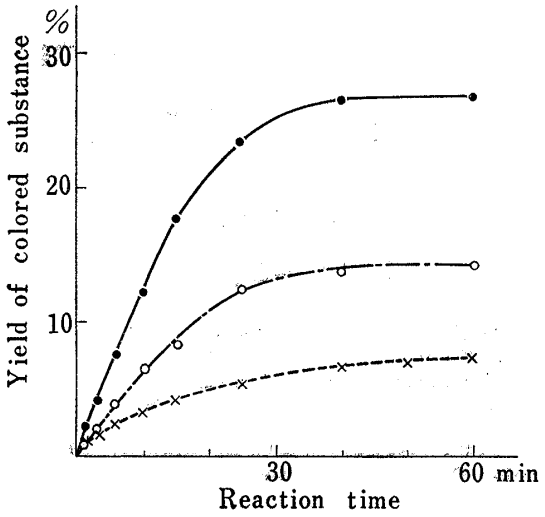
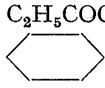
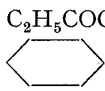
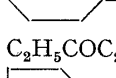


Fig. 2. The Rate of the Zimmermann Reaction with Cyclopentanone (—•—), Cyclohexanone (—○—) and Diethyl Ketone (··· × ···)

On the other hand Zimmermann reaction was carried out with these simple ketones according to the method of Callow, *et al.*[9] The extinction value of the colored solution produced was measured at absorption maximum and the apparent yield of the Zimmermann chromophore, calculated from their molar extinction coefficient, was plotted against the incubation time (see Fig. 2). The initial velocity of the color development with cyclopentanone was distinctly much faster than those with the others. It is of particular interest that the sequential order of the rate of Zimmermann reaction is in qualitative agreement with that of deuterium exchange reactivity. This parallelism suggests that with these simple ketones the rate-determining step of Zimmermann reaction would exist in deprotonation of the active methylene group.

Table II. The Rate of the Base-catalyzed Deuterium Exchange of Cyclopentanone, Cyclohexanone and Diethyl Ketone

Combinations of compounds			Reaction conditions	Rate of deuterium exchange[a]				
				Reaction time (hr)				
No.	Compounds	Amounts (mg)		1	2	3	4	5
1	$C_2H_5COC_2H_5$	14.73	0.2% K_2CO_3	+				+++
		14.96	at 80°	++				+++
2	$C_2H_5COC_2H_5$	13.69	0.4% K_2CO_3	+	+	+·∴·	++	++·∴·
		14.93	at room temp.	+	+	+·∴·	++·∴·	++·∴·
3	$C_2H_5COC_2H_5$	14.66	0.08% K_2CO_3			+		
		13.46	at room temp.		+++			

a) +: 10—40%, ++: 40—70%, +++: 70—100%

8) Y. Kawazoe and M. Ohnishi, *Chem. Pharm. Bull.* (Tokyo), **12**, 846 (1964); *Idem*, *ibid.*, **14**, 1413 (1966).
9) N.H. Callow, R.K. Callow and C.W. Emmens, *Biochem. J.*, **32**, 1312 (1938).

Studies on deuterium exchange were further extended to 17- and 16-oxosteroids. The androstanones were dissolved in pyridine–deuterium oxide containing potassium carbonate and tetramethylammonium bromide, an internal standard. The sample solution being incubated, the amount of water liberated was likewise estimated along with the reaction time by means of nuclear magnetic resonance spectrometry. The results thus obtained were illustrated in Fig. 3. It seems very likely that with androstan-16-one one of the four hydrogens attached to α-carbons is readily exchanged and the most inert one is not displaced with deuterium under these conditions.[10] The remaining two hydrogens of the active methylenes exhibited the same degree of reactivity as those of cyclopentanone and 17-oxosteroid. However, the Zimmermann reaction of these compounds was found to be quite different from their deuterium exchange reactivity mentioned above. As can be seen in Fig. 4, cyclopentanone exhibited Zimmermann coloration much faster than either of these two oxosteroids.

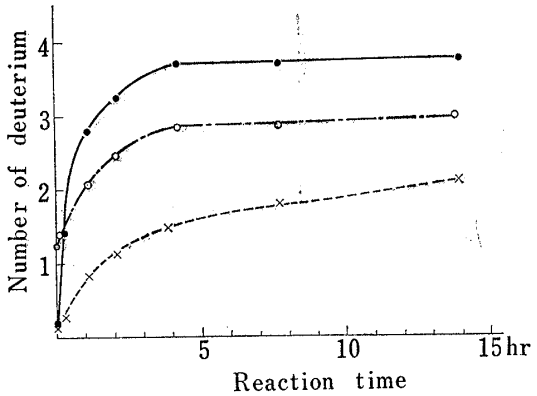


Fig. 3. The Rate of the Base-catalyzed Deuterium Exchange of Cyclopentanone (—•—), 3β-Hydroxyandrostan-16-one (---o---) and 3β-Hydroxyandrostan-17-one (···×···)

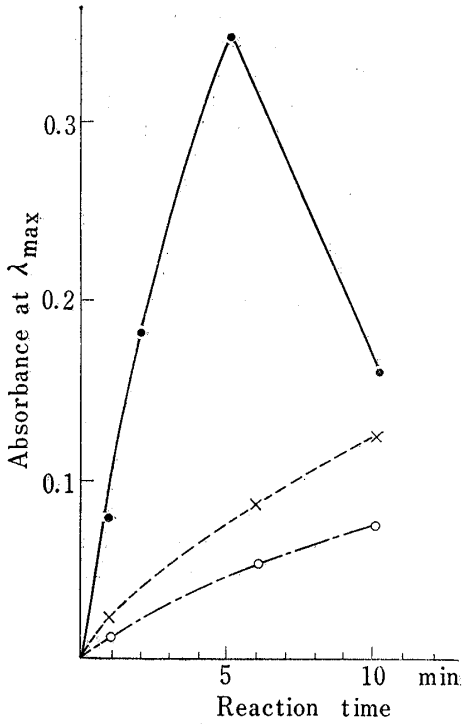


Fig. 4. The Rate of the Zimmermane Reaction with Cyclopentanonn (—•—), 3β-Hydroxyandrostan-16-one (---o---) and 3β-Hydroxyandrostan-17-one (··· × ···) (0.2 μmole each)

It is to be noted that the close relationship between deuterium exchange reactivity and Zimmermann reaction was observed with some simple ketones, while the correlation of these reactions was not necessarily effective with the ketones in the fused ring system such as oxosteroids. This discrepancy may be ascribable to the difference in steric factor against the access of the reagent toward the carbanion. The rate of Zimmermann color development with various steroidal ketones seems to depend on not only the relative ease of deprotonation of α-methylene but also the steric requirement of bulky 2,4-dinitrophenyl group to be introduced.

Experimental[11]

Isolation of Zimmermann Complex——i) From Cyclopentanone: To a solution of cyclopentanone (1 g) and *m*-dinitrobenzene (2 g) dissolved in EtOH (100 ml) was added ethanolic 2.5 N KOH (5 ml) dropwise under stirring, and the reaction mixture was allowed to stand in refrigerator for 30 min. Upon evaporation of solvent a dark brown solid residue was obtained. The crude product was dissolved in EtOH and passed through a column of acid-washed Al_2O_3 (80 g). The yellow-colored fraction, which showed distinct

10) It is supposed that the most active hydrogen would be 17α-H and the least one 15β-H considering the interaction with C-13-methyl and neighboring hydrogens.

11) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise stated.

Zimmermann color promptly upon addition of NaOH solution, was collected and concentrated to dryness *in vacuo* below 25° to provide yellow solid product. This crude product was dissolved in hexane–benzene (1:3) and rechromatographed on acid–washed Al_2O_3 (10 g). Elution with benzene afforded pale yellow crystalline product. Recrystallization from MeOH gave 2-(2,4-dinitrophenyl)cyclopentanone (29.4 mg) as pale yellow needles. mp 116—118°. *Anal.* Calcd. for $C_{11}H_{10}O_5N_2$: C, 52.80; H, 4.03; N, 11.20. Found: C, 52.80; H, 4.16; N, 10.99.

ii) From Diethyl Ketone: To a solution of *m*-dinitrobenzene (520 mg) dissolved in diethyl ketone (6.8 g) was added ethanolic 2.5 N KOH (1.3 ml) dropwise under stirring, and the reaction mixture was allowed to stand for 10 min under cooling in ice–water. The reaction mixture was diluted with EtOH (20 ml) and passed through a column of acid–washed Al_2O_3 (25 g). The reaction product was treated in the same manner as described above. Elution with hexane–benzene (1:2) and recrystallization of the eluate from MeOH gave 2-(2,4-dinitrophenyl)pentan-3-one (23.7 mg) as pale yellow needles. mp 91.5—92°. *Anal.* Calcd. for $C_{11}H_{12}O_5N_2$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.74; H, 4.93; N, 11.12.

iii) From Cholestan-3-one: To a solution of cholestan-3-one (2 g) and *m*-dinitrobenzene (0.87 g) dissolved in EtOH (120 ml) was added ethanolic 2.5 N KOH (3.2 ml) dropwise under stirring, and the reaction mixture was allowed to stand in refrigerator overnight. The reaction product was treated in the same manner as described above. Elution with hexane–benzene (2:3 to 1:1) and recrystallization of the eluate from EtOH gave 2*a*-(2,4-dinitrophenyl)cholestan-3-one (216 mg) as colorless needles. mp 178—180°, $[\alpha]_D^{22.8}$ +3° (c=1.18). *Anal.* Calcd. for $C_{33}H_{48}O_5N_2$: C, 71.71; H, 8.75; N, 5.07. Found: C, 72.14; H, 8.58; N, 5.34.

2-(2,4-Dinitrophenyl)cyclohexanone——Prepared from cyclohexanone pyrrolidyl enamine and 2,4-dinitrochlorobenzene according to the method of Kuehne. mp 92—94° (Reported mp 99—100°).[4b]

Measurement of Zimmermann Reaction Velocity——i) With Cyclopentanone, Cyclohexanone and Diethyl Ketone: The Zimmermann reaction was carried out according to the procedure of Callow, *et al.*[9] with use of 0.2 μmole each of sample, 2% ethanolic solution of *m*-dinitrobenzene and ethanolic 0.25 N KOH solution. The reaction mixture was incubated for 1,2,5 and 10 min, respectively and the absorption spectra were obtained by Hitachi Model EPS–2U auto–recording spectrophotometer with scanning time of 1 min. The absorbance (Ax) of the colored solution produced was measured at λ_{max}, and the apparent yield (Yx) of the Zimmermann chromophore, which was calculated from the molar concentration of the sample solution (Cx) and its molar extinction coefficient (Ex) according to equation (1), was plotted against the incubation time.

$$Y_x(\%)=\frac{A_x/C_x}{\varepsilon_x}\times 100 \qquad (1)$$

where $C_x=0.2\times 10^{-6}$

ii) With Oxosteroids: The Zimmermann reaction was carried out in the same manner as described above with use of 0.2 μmole each of cyclopentanone, 3β-hydroxyandrostan-17-one and 3β-hydroxyandrostan-16-one and of ethanolic 2.5N KOH solution. Extinction values at λ_{max} observed were plotted against the incubation time.

Determination of Base-catalyzed Deuterium Exchange Reactivity——The NMR spectra were obtained by Japan Electron Optics Model JNM–3H–60 spectrometer at 60 Mcps. The signal integrations were carried out with JES–1D integrator attached to the spectrometer.

i) Of the Simple Ketones: Two sets of combination of ketones, diethyl ketone and cyclopentanone, diethyl ketone and cyclohexanone, were chosen. An internal standard, *tert*-BuOH (139 mg), and K_2CO_3 (40, 20, 8 mg) were dissolved in D_2O–CH_3OD (3:7) so that the whole volume was brought to 10 ml. A 0.5 ml aliquot of this solution was transferred into the tube to dissolve a pair of samples to be examined. The tube was sealed, and deuterium exchange reaction was carried out under the conditions as given in Table II. The NMR spectral measurements of the reaction mixture were run along with the reaction time, and the areal intensity of the active methylene peak referring to that of the internal standard was measured. The rate of hydrogen exchanged with deuterium at definite elapsed time was estimated by comparison with the areal intensity obtained at the starting time.

ii) Of the Oxosteroids: An internal standard, $(CH_3)_4NBr$ (300 mg), was dissolved in 0.2% K_2CO_3–D_2O solution–pyridine (2:3) so that the whole volume was brought to 25 ml. With a 0.5 ml aliquot the sample accurately weighed was dissolved, and the deuterium exchange reaction was carried out at 70° together with the blank test. The areal intensities of H_2O and internal standard peaks were measured in the same manner as in i). The number (x') of hydrogens exchanged with deuterium was calculated according to equation (2).

$$x'=0.468(\beta-\alpha)\times Mb/b \qquad (2)$$

where α: Ratio in the areal intensity of H_2O peak to the reference hydrogen peak determined with the blank

β: Ratio in the areal intensity of H_2O peak to the reference hydrogen peak determined with the sample

Mb: Molecular weight of the sample

b: Weight of the sample taken (mg)

The number (x) of hydrogens of the active methylenes exchanged with deuterium was obtained by substracting the number of hydroxyl group present in the sample molecule from x'.

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