UDC 547.92·07

39. Katsumi Tanabe, Yasuhiro Morisawa, and Rinji Takasaki : Steroid Series. X.[1] Ozonolysis of 7-Oxo-Δ^5-Steroids. An Attempt to synthesize 6-Azasteroid Hormone Analogs.

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Since the structures of the naturally occurring steroid hormones were established, extensive efforts have been devoted during the past decade to study the effects of their structural modification upon biological activities. As a part of investigations along this line numerous reports concerning the synthesis of oxa- and aza-steroids have been published by a number of workers.[2] The literatures shows that preparation of the majority of aza-steroids involves insertion of a nitrogen atom into a ring of the carbon skeleton of the steroid nucleus, with a result of expansion of the ring from a six- to a seven-membered and in the case of ring D from a five- to a six-membered ring.

In view of a biological significance of the over-all shape of the molecule upon the enzymatic actions, it seemed of interest to prepare steroid derivatives containing a six-membered B-ring whose C_6-carbon atom is substituted with oxygen or nitrogen and possessing nearly the same order of the molecular dimensions as natural steroids. For this purpose a steroidal 5-oxo-5,7-seco-B-nor-7-carboxylic acid(II)was utilized as a starting material, which could be obtained by ozonolysis of a 7-oxo-Δ^5-steroid derivative (I).

While this work was in progress, literatures dealing with a similar project appeared from several groups of workers;[2~5] Jacobs and Brownfield described the preparation of 6-oxa- and 6-aza-steroid derivatives in the cholestane series by essentially the same sequence as ours.[2] Therefore, the present paper is limited to the description of the structure of a neutral product, isolated along with a B-nor seco acid (II) when 7-oxo-Δ^5-steroid compounds were subjected to ozonolysis.

Ozonization of 7-oxocholesterol acetate (Ia) in a mixture of acetic acid and ethyl acetate according to the method of Turner[6] and Dauben,[7] followed by oxidation with hydrogen peroxide and chromatographic separation of the products over silica gel, yielded a neutral product IIIa of m.p. 147°, as well as a keto acid, m.p. 167°, $[\alpha]_D$ +73°, in 31% and 28% yield, respectively.[8] Although the carboxylic acid showed a higher melting point than that of 3β-acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oic acid (IIa), m.p. 159.5°, $[\alpha]_D$ +71±2° reported by the American workers, both acids were assumed to be identical since they afforded the same methyl esters melting at 93° with $[\alpha]_D$ +69°(lit.[2] m.p. 92°, $[\alpha]_D$ +67±2°). The acid (IIa) seemed to exist prior to chromatography in the pseudo acid form because it remained in the organic solvent even after fractionation with an aqueous alkali.[9]

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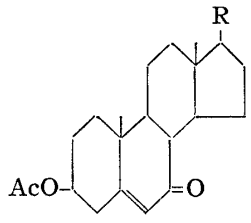
1) Part IX : This Bulletin, **10**, 1177 (1962).
2) T.L. Jacobs, R.B. Brownfield : J. Am. Chem. Soc., **82**, 4033 (1960). A Comprehensive list of references to the field of heterocyclic steroid derivatives is presented therein.
3) H. Lettre, L. Knof : Ber., **93**, 2860 (1960).
4) N.W. Atwater : J. Am. Chem. Soc., **83**, 3071 (1960).
5) J.P. Kutney, R.A. Johnson : Chem. & Ind. (London), **1961**, 1713.
6) R.B. Turner : J. Am. Chem. Soc., **72**, 579 (1950).
7) W.G. Dauben, K.H. Takemura : *Ibid.*, **75**, 6302 (1953).
8) An improved procedure for the preparation of methyl 3β-acetoxy-17β-benzoyloxy-5-oxo-5,7-seco-B-norandrostan-7-oate (cf. II) in 73% yield by ozonolysis of 3β-acetoxy-17β-benzoyloxyandrost-5-en-7-one (cf. I) in methylene chloridemethanol was described by N.W. Atwater.[4]
9) M. Uskokovic, R.I. Dorfman, M. Gut : J. Org. Chem., **23**, 1947 (1958).

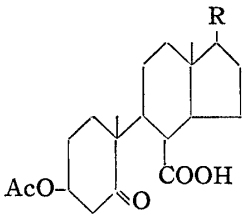
The neutral product IIIa, which Jacobs and Brownfield had not encountered, was found to be non-peroxidic as shown by a negative test with potassium iodide and it was isolated also by direct chromatography of the ozonized mixture. Mild treatment of this compound with a mixture of acetic acid and ethyl acetate furnished the keto acid IIa in good yield. The analytical values of the neutral compound corresponded to a composition of $C_{29}H_{46}O_6$, indicating that three atoms of oxygen had been incorporated into the parent compound. Its infrared absorption bands at 1773 and 1745 cm^{-1} in carbon tetrachloride solution, and 1757 and 1734 cm^{-1} in nujol mull suggested the presence of either a five-membered lactonic carbonyl group or a six-membered one with a somewhat higher frequency.

Similar spectral features in the carbonyl region were also displayed by a pseudo acid acetate, 3β,5-diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa), which was derived from the δ-keto acid (IIa) in a fair yield on heating it in acetic anhydride solution : The acetate IVa exhibited infrared absorption bands at 1777 and 1745 cm^{-1} in carbon tetrachloride, and 1765, 1757 (doublet) and 1733 cm^{-1} in nujol mull. The structure of IVa was based on its analytical values as well as the well established fact that γ- and δ-keto carboxylic acids on treatment with acetic anhydride are converted with ease into the corresponding pseudo acid acetates.

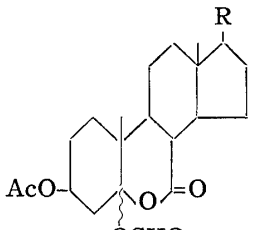
Treatment of the compound IIIa with an alcoholic sodium hydroxide solution and titration of the reaction mixture revealed that three equivalent moles of alkali had been consumed. On acidification of the mixture there was obtained an acid, which was proved to be identical with the known 5-oxo-5,7-seco-B-norcholest-3-en-7-oic acid (Va) obtainable from 3β-acetoxy-5-oxo-5, 7-seco-B-norcholestan-7-oic acid (IIa) by a mild alkaline treatment.[2] The same unsaturated acid Va was derived also from the



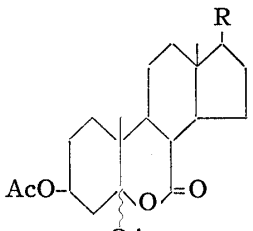
Ia, b, c



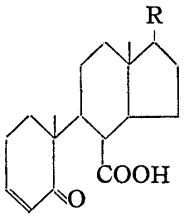
IIa, b, c



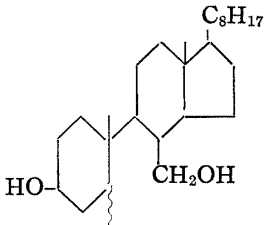
IIIa, b, c



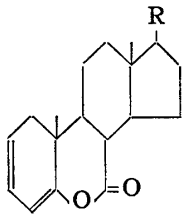
IVa, b



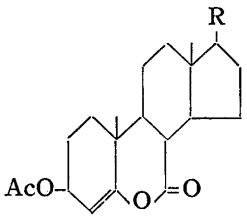
Va, b



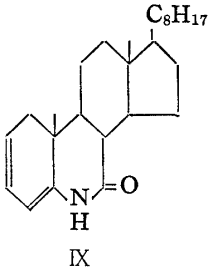
VI



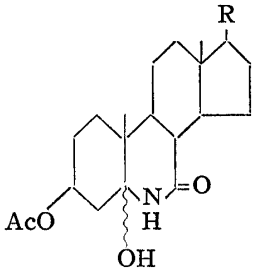
VIIa, b, c



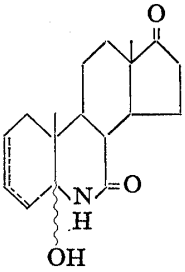
VIIIa, b



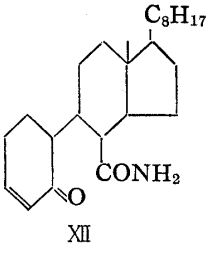
IX



Xb, c



XI



XII

a; R=C_8H_{17}. b; R=O. C; R=$COCH_3$.

pseudo acid acetate IVa by a similar treatment. The titration values, therefore, correspond to the sum of the titration of the carboxylic acid Va, acetic acid, and another acidic substance. This implies that the compound IIIa contains, besides a 3β-acetoxyl group, a substituent liberating an acid in an alkaline medium.

On the basis of the above-described observations, together with the similar chemical behaviors between the pseudo acid acetate IVa and the neutral compound IIIa as shown below, the structure of the latter was assumed to be 3β-acetoxy-5-formoxy-6-oxa-5ξ-cholestan-7-one and this assignment eventually was confirmed by the isolation of the compound, together with other products, upon treatment of the δ-keto acid (IIa) with acetic-formic anhydride (*vide infra*). The absorption of the lactonic carbonyl group of the compounds (IIIa and IVa) at a higher frequency than that of the normal six-membered lactone may be ascribed to the electronegativity of the acyl group at 5-position.[10]

Reduction of the methyl ester of the acid IIa with lithium aluminum hydride afforded 3β,5,7-trihydroxy-5,7-seco-B-nor-5ξ-cholestene (VI), which could be derived also from the pseudo acid esters (IIIa and IVa) on reduction with the same reagent.

The keto acid IIa, when refluxed in acetic anhydride solution containing small amounts of anhydrous sodium acetate, yielded, along with a smount of 3β-acetoxy-6-oxacholest-4-en-7-one (VIIIa), 6-oxacholesta-2,4-dien-7-one (VIIa) of m.p. 117°, which exhibited absorption maxima at 273 mμ (ε 8,400) in the ultraviolet, and at 1755 (lactone CO), 1663, and 1595 cm^{-1} ($\Delta^{2,4}$) in the infrared region. The compound VIIIa displayed an infrared band at 1670 cm^{-1} due to the carbon-carbon double bond characteristic of an enol lactone.[11] When the above reaction was conducted under gentle heating on a steam bath, nearly the same quantities of the enol lactone VIIIa and the pseudo acid acetate IVa were produced. The doubly unsaturated lactone VIIa was found to be formed also by pyrolytic decomposition of either the formate IIIa or the acetate IVa.

Treatment of the δ-keto acid (IIa) with acetic-formic anhydride and subsequent chromatographic separation of the product afforded, along with the acetate IVa and the enol lactone VIIIa, the pseudo acid formate IIIa melting at 145°, which was identical in all respects with that obtained on ozonolysis of Ia.

When 6-oxacholesta-2,4-dien-7-one (VIIa) was treated with gaseous ammonia in benzene solution according to the procedure, by which Uskokovic and Gut successfully synthesized 4-azasteroid derivatives,[12] there was obtained 6-azacholesta-2,4-dien-7-one (IX), whose physical constants were in good agreement with those reported by Jacobs and Brownfield.[2] By the same method the pseudo acid esters (IIIa and IVa) yielded the identical 6-aza derivative IX, but unexpectedly the enol lactone VIIIa gave 5-oxo-5,7-seco-B-norcholest-3-en-7-oic amide (XII) of m.p. 204° (decomp.).

Ozonolysis of 3β-acetoxyandrost-5-en-7,17-dione (Ib) proceeded analogously as in the cholestane series to give the corresponding 5,7-seco acid (IIb) of m.p. 177° (decomp.) and its pseudo acid formate IIIb melting at 205° with decomposition. In this case the compound IIb, in contrast with the cholestane series, was extracted as an acid form from the reaction mixture. Alkaline treatment of either the keto acid IIb or the formate IIIc afforded 5,17-dioxo-5,7-seco-B-norandrost-3-en-7-oic acid (Vb). On chromatography over neutral alumina, the methyl ester of IIb was partly converted into the methyl ester of the acid Vb with elimination of a 3β-acetoxyl group.

The δ-keto acid (IIb), on heating in acetic anhydride solution at 80~90°, yielded 3β,5-diacetoxy-6-oxa-5ξ-androstan-7,17-dione (IVb) of m.p. 185°. The acid IIb afforded

10) R. B. Woodward, E. G. Kovach: J. Am. Chem. Soc., **72**, 1009 (1950); W. Brügel, G. Stengel, F. Reicheneder, H. Suter: Angew. Chem., **68**, 441 (1956).
11) H. Rosenkrantz, M. Gut: Helv. Chim. Acta., **36**, 1000 (1953).
12) M. Uskokovic, M. Gut: *Ibid.*, **42**, 2258 (1959).

6-oxaandrosta-2,4-dien-7,17-dione (VIIb) on treatment either with a mixture of acetic anhydride and acetyl chloride or with acetic anhydride containing anhydrous sodium acetate, under the latter reaction conditions a small amount of 3β-acetoxy-6-oxaandrost-4-en-7,17-dione (VIIIb) was also isolated.

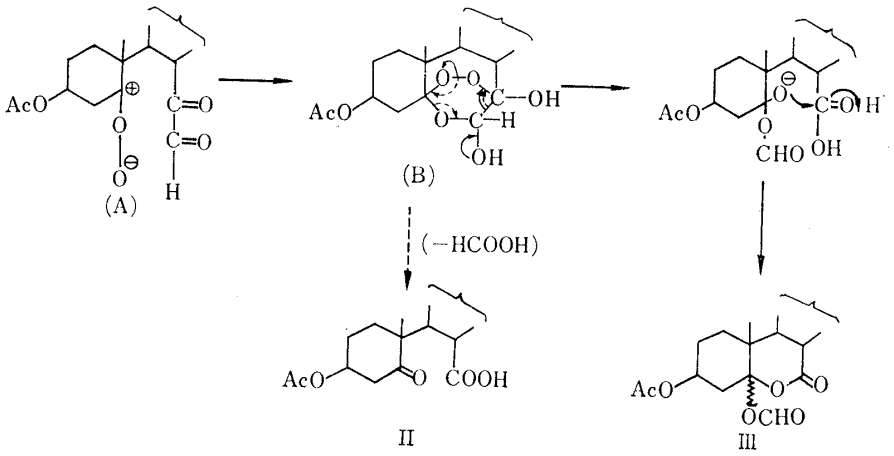
On refluxing in acetic-formic anhydride solution the acid IIb was converted into a mixture, which on chromatography was separated into the doubly unsaturated enol lactone VIIb, 3β-acetoxy-enol lactone (VIIIb), the pseudo acid acetate IVb, and the formate IIIb. The last compound was identical in all respects with that formed on ozonolysis of Ib.

On treatment with gaseous ammonia in benzene solution, in contrast to the cholestance series, the formate IIIb and the acetate IVb afforded 3β-acetoxy-5-hydroxy-6-aza-5ξ-androstan-7,17-dione (Xb), and 6-oxaandrosta-2,4-dien-7,17-dione (VIIb) was converted into 5-hydroxy-6-aza-5ξ-androst-2 or 3-en-7,17-dione (XI). The pseudo amide Xb was unstable and turned into a brown syrupy material within a day on standing at room temperature. The structures of Xb and XI were deduced from the analytical values as well as examinations of their infrared spectra (cf. experimental part); the formation of such a pseudo amide was also reported in the literature.[12]

3β-Acetoxypregn-5-en-7,20-dione (Ic), on ozonolysis, afforded 3β-acetoxy-5-formoxy-6-oxa-5ξ-pregnan-7,20-dione (IIIc) of m.p. 153° (decomp.), and an oily acidic substance. Although the latter compound failed to crystallize, it was most likely an impure keto acid IIc, because it yielded 6-oxapregna-2,4-dien-7,20-dione (VIIc) of m.p. 159° when heated in acetic anhydride containining anhydrous sodium acetate.

The formate IIIc on treatment with ammonia in benzene, gave 3β-acetoxy-5-hydroxy-6-aza-5ξ-pregnan-7,20-dione (Xc) of m.p. 127.5°, whose infrared spectrum showed bands at 3450 (OH), 3230, 3100 (NH), 1725 (AcO), 1708 (20-CO), and 1655 cm^{-1} (CONH).

The concurrent formation of the seco-nor keto acid II and its pseudo acid formate III by an abnormal ozonization of 7-oxo-Δ^5-steroid would be explained by postulating an intermediate compoud (B), one of the probable formulations,[13,14] which may undergo the intramolecular rearrangement indicated below.



The present sequence for the synthesis of 6-aza-steroid retaining an oxygen function at 3-position encountered difficulties due to the ready elimination of the 3β-acetoxyl group located at the β-position with respect to the 5-carbonyl substituent of the intermediates. Another promising route is now under investegation.

13) R. Criegee, A. Kerckow, H. Zinke : Ber., 88, 1883 (1955); R. Criegee, G. Blust, G. Lohaus : Ann., 583, 5 (1953); R. Criegee : *ibid.*, 583, 1 (1953).
14) cf. W.G. Dauben, H.G. Wight, G.A. Boswell : J. Org. Chem., 23, 1787 (1958).

Experimental*2

Ozonolysis of 3β-Acetoxycholest-5-en-7-one (Ia)——A solution of 12 g. of Ia in a mixture of 400 cc. of AcOH and 400 cc. of AcOEt was treated with an ozonized air (0.036 mole of O_3/hr.) at 0° for 4 hr. To the reaction mixture was added 120 cc. of 3% H_2O_2, the solution vigorously stirred for 20 min., and left standing for 30 hr. at room temperature. The mixture was diluted with 500 cc. of AcOEt and fractionated with an ice-cold 1% NaOH solution. The organic layer was washed with water until neutral, dried, and the solvent removed under reduced pressure to give 7.9 g. of a syrupy residue, which was chromatographed over 350 g. of silica gel.

The eluate with benzene-$CHCl_3$ (1 : 1) afforded 4.1 g. (31 %) of 3β-acetoxy-5-formoxy-6-oxa-5ξ-cholestan-7-one (IIIa), which after recrystallization from hexane gave needles, m.p. 146~147°, $[\alpha]_D^{28}$ −19° (c=3.05). *Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found : C, 70.99; H, 9.50. IR $\lambda_{max}^{CCl_4}$ cm^{-1} : 1773 (lactone CO), 1745 (AcO, HCOO); λ_{max}^{Nujol} cm^{-1} : 1757 (lactone CO), 1734 (AcO, HCOO).

Elution with $CHCl_3$ containing 2% MeOH afforded 3.5 g. (28 %) of 3β-acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oic acid (IIa) melting at 163~165°, which on further recrystallizations from hexane gave needles, m.p. 166~167°, $[\alpha]_D^{28}$ +73° (c=3.1) (lit.[2] m.p. 159~159.5°, $[\alpha]_D^{26}$ +71±2°). *Anal.* Calcd. for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found : C, 72.55; H, 9.99.

The methyl ester of IIa prepared with CH_2N_2 had m.p. 92~93°, $[\alpha]_D^{27}$ +69° (c=2.10) (lit.[2] m.p. 91~92°, $[\alpha]_D^{26}$ +67±2°). *Anal.* Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15. Found : C, 73.16; H, 9.98. IR $\lambda_{max}^{CCl_4}$ cm^{-1} : 1732 (AcO, CO_2Me), 1715 (5-CO).

A solution of 500 mg. of the formate IIIa in a mixture of 5 cc. of AcOH and 5 cc. of AcOEt was warmed at 60~70° for 1 hr. The solution was concentrated to a small volume under reduced pressure, whereby 420 mg. of white needles, m.p. 156~157°, deposited. After recrystallization from hexane-benzene the m.p. was raised to 167~168°. The IR spectrum was identical with that of the acid IIa obtained above.

3β,5-Diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa)——A solution of 1 g. of 3β-acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oic acid (IIa) in 10 cc. of Ac_2O was heated on a steam-bath for 1 hr. The Ac_2O was removed from the reaction mixture under reduced pressure to give a syrupy substance, which was chromatographed over 30 g. of silica gel. The eluate with benzene gave 60 mg. of 3β-acetoxy-6-oxa-cholest-4-en-7-one (VIIIa) of m.p. 134~136°, $[\alpha]_D^{28}$ +10.1° (c=2.10) after recrystallization from hexane. *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found : C, 75.31; H, 9.87. IR λ_{max}^{Nujol} cm^{-1} : 1740 (AcO, lactone CO), 1670 (Δ^4).

Elution with benzene-$CHCl_3$ (1 : 1) afforded 640 mg. of a white crystalline substance, which after recrystallization from hexane yielded 3β,5-diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa) as needles, m.p. 146~147°, $[\alpha]_D^{27}$ +52° (c=2.385). *Anal.* Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found : C, 71.20; H, 9.75. IR $\lambda_{max}^{CCl_4}$ cm^{-1} : 1777 (lactone CO), 1745 (AcO); λ_{max}^{Nujol} cm^{-1} : 1765, 1757 (lactone CO), 1733 (AcO).

5-Oxo-5,7-seco-B-norcholest-3-en-7-oic Acid (Va)——(i) 3β-Acetoxy-5-formoxy-5ξ-cholestan-7-one (IIIa; 500 mg.) was dissolved in a mixture of 10 cc. of EtOH and 10 cc. of 0.4565*N* NaOH solution and the mixture was refluxed for 1 hr. on a steam-bath. The solution was ditued with 50% EtOH to 100 cc. Titration of a 10 cc. portion of this solution required 1.720 cc. of 0.982*N* HCl, corresponding to three equivalents of free carboxylic acid.

The neutralized solution was acidified with AcOH and concentrated to a small volume, to which water was added to precipitate a white product. It was extracted with AcOEt and the extract washed with water, dried, and the solvent evaporated to leave a crystalline residue, which on recrystallization from hexane gave 5-oxo-5,7-seco-B-norcholest-3-en-7-oic acid (Va) as needles, m.p. 176~177°, $[\alpha]_D^{29}$ +87° (c=2.665) (lit. m.p. 164~165°, $[\alpha]_D$ +81±1°[2] ; m.p. 183~184°, $[\alpha]_D$ +79°[5]). *Anal.* Calcd. for $C_{26}H_{42}O_3$: C, 77.56; H, 10.52. Found : C, 77.03; H, 10.52. IR λ_{max}^{Nujol} cm^{-1} : 1725 (COOH), 1675 (Δ^3-5-CO). UV λ_{max}^{EtOH} : 225.2 mμ (ε 7,750).

(ii) A suspension of 500 mg. of 3β-acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oic acid (IIa) in 2*N* NaOH solution was heated for 30 min. on a steam-bath or allowed to stand overnight at room temperature, during which time the crystals went into solution. The mixture was acidified with AcOH and extracted with AcOEt. The extract was dried and the solvent removed to give a semi-solid residue, which was chromatographed over 15 g. of silica gel. Elution with $CHCl_3$ containing 5% MeOH gave 5-oxo-5,7-seco-B-norcholest-3-en-7-oic acid (Va) of m.p. 173° (from hexane), which was proved to be identical with the sample obtained above by mixed m.p. determination and direct comparison of the IR spectra.

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*2 All m.p.s were uncorrected. Rotations were measured in $CHCl_3$ solution. Infrared spectra were determined using a Perkin-Elmer Model 21 or Infracord spectrophotometer. A Perkin-Elmer Model 221 Prism-Grating spectrophotometer was used for the determination of the carbonyl absorption bands of the pseudo acid esters (III and IV).

(iii) 3β,5-Diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa) was treated with NaOH solution in the same manner as IIIa, and the product obtained was chromatographed on silica gel to give Va of m.p. 174～175°, which showed no depression of m.p. on admixture with the acid obtained above.

3β,5,7-Trihydroxy-5,7-seco-B-nor-5ξ-cholestane (VI)——(i) A solution of 1.5 g. of methyl 3β-acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oate (cf. IIa) in 30 cc. of dry Et_2O was added dropwise to a suspension of 1.2 g. of $LiAlH_4$ in 100 cc. of dry Et_2O during 20 min. The mixture was stirred at room temperature for 2.5 hr., and the excess reagent was decomposed by addition of aq. AcOH. The organic layer which separated was washed with 1% $NaHCO_3$, dried, and the solvent removed to give 1.42 g. of a crystalline powder melting at 196～198°. Repeated recrystallization from hexane-benzene gave 3β,5,7-trihydroxy-5,7-seco-**B**-nor-5ξ-cholestane (VI) of m.p. 208～209°. *Anal.* Calcd. for $C_{26}H_{48}O_3$: C, 76.41; H, 11.84. Found : C, 76.56; H, 11.58.

(ii) A solution of 500 mg. of 3β-acetoxy-5-formoxy-6-oxa-5ξ-cholestan-7-one (IIIa) in 10 cc. of dry Et_2O was added dropwise to a solution of 150 mg. of $LiAlH_4$ in 30 cc. of dry Et_2O at room temperature. The mixture was stirred for 2.5 hr. and the excess reagent was decomposed with aq. AcOH. The Et_2O layer was worked up in the usual way. The product was repeatedly recrystallized from hexane-benzene to give the triol VI as plates, m.p. 206～207°.

6-Oxacholesta-2,4-dien-7-one (VIIa) and 3β-Acetoxy-6-oxacholest-4-en-7-one (VIIIa)——(i) A solution of 3.0 g. of 3β-acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oic acid (IIa) in 30 cc. of Ac_2O containing 30 mg. of freshly fused AcONa was refluxed for 4 hr. under a N_2 atmosphere. The solvent was then concentrated to a small volume below 50° under reduced pressure. To the residue was added water and the product was taken up in AcOEt. The extract was washed with 2% $NaHCO_3$ solution, dried, and the solvent removed. The residue was chromatographed over 90 g. of silica gel. The first eluate with benzene afforded 2.21 g. of 6-oxacholesta-2,4-dien-7-one (VIIa), which showed m.p. 116～117, $[\alpha]_D^{29}$ +68° (c=3.695) after recrystallization from hexane. *Anal.* Calcd. : for $C_{26}H_{40}O_2$: C, 81.20; H, 10.48. Found : C, 81.03; H, 10.28. IR λ_{max}^{Nujol} cm^{-1} : 1755 (lactone CO), 1663, 1595 ($\Delta^{2,4}$). UV λ_{max}^{EtOH} : 273 mμ (ε 8,400).

The second fraction with the same solvent gave 150 mg. of 3β-acetoxy-6-oxacholest-4-en-7-one (VIIIa; see above) as needles, m.p. 137～138° after recrystallization from hexane.

The third eluate with $CHCl_3$ containing 2% MeOH gave 5-oxo-5,7-seco-B-norcholest-3-en-7-oic acid (Va) as needles, m.p. 171～172° (from hexane), whose IR spectrum was identical with that of the sample described above.

(ii) A mixture of 1.0 g. of IIa, 25 mg. of freshly fused AcONa, and 10 cc. of Ac_2O was heated on a steam-bath for 2.5 hr., and the solvent was removed under reduced pressure. The residue was chromatographed over 30 g. of silica gel. The eluate with benzene afforded 270 mg. of the enol lactone VIIIa, m.p. 137～138° (from hexane), undepressed on admixture with the sample described above. The fraction eluted with benzene-$CHCl_3$ (8 : 2) gave 273 mg. of 3β,5-diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa) which after recrystallization from hexane melted at 148°.

(iii) 3β-Acetoxy-5-formoxy-6-oxa-5ξ-cholestan-7-one (IIIa; 100 mg.) was heated at 160～170° under 4～5×10^{-4} mm. Hg pressure for 1 hr. The product was dissolved in Et_2O and the solution was washed with 2% $NaHCO_3$ solution. Removal of the solvent yielded 91 mg. of a neutral substance, which was chromatographically purified using 3 g. of silica gel. The fraction eluted with benzene gave 64 mg. of VIIa of m.p. 114～116° (from hexane), which was identical in all respects with the sample obtained above.

(iv) 3β,5-Diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa; 100 mg.) was pyrolyzed in the same manner as IIIa, and the product, after chromatography over silica gel and recrystallization from hexane, gave 56 mg. of VIIa of m.p. 116～118°.

Treatment of 3β-Acetoxy-5-oxo-5,7-seco-B-norcholestan-7-oic Acid (IIa) with Acetic-formic Anhydride——A solution of 500 mg. of the acid IIa in 5 cc. of acetic-formic anhydride (b.p_{20} 30～32°) prepared from Ac_2O and anhyd. HCOOH[15] was allowed to stand for 4 days at room temperature and then warmed on a steam-bath for 20 min. Water and AcOEt were added to the solution, the mixture was kept for 2 hr. at room temperature, washed with 2% $NaHCO_3$ solution, dried, and the solvent was removed under reduced pressure to leave a syrupy substance, which was chromatographed over 10 g. of silica gel in benzene.

The first fraction gave 186 mg. of 6-oxacholesta-2,4-dien-7-one (VIIa), m.p. 114～116° (from hexane), identical with the sample obtained above. The second fraction yielded 50 mg. of 3β,5-diacetoxy-6-oxa-5ξ-cholestan-7-one (IVa), m.p. 142° (from hexane), whose identity was confirmed by admixture and IR comparison with the sample obtained above. The third fraction afforded 67 mg. of a substance melting at 140～142°, which was rechromatographed over 2.5 g. of silica gel. The benzene eluate yielded 3β-acetoxy-5-formoxy-6-oxa-5ξ-cholestan-7-one (IIIa), m.p. 145° (from hexane), undepressed on admixture with the sample obtained on ozonolysis of 7-oxocholesterol acetate (Ia), and the IR spectra of both compounds were completely identical.

15) K. Freudenberg, W. Jacob : Ber., **80**, 325 (1947).

6-Azacholesta-2,4-dien-7-one (IX)——(i) A solution of 500 mg. of 6-oxacholesta-2,4-dien-7-one (VIIa) in anhyd. benzene was bubbled with a dry NH_3 for 2 hr. at room temperature and the solution was left standing overnight. The solvent was removed to give a semi-solid residue, which was chromatographed over silica gel. The benzene eluate yielded 110 mg. of 6-azacholesta-2,4-dien-7-one (IX), which on recrystallization from 95% EtOH showed m.p. 187~189° (decomp.), $[\alpha]_D^{27}$ +47° (c=2.285) (lit.[2] m.p. 188~189° (decomp.), $[\alpha]_D^{27}$ +49±3°). *Anal.* Calcd. for $C_{26}H_{41}ON$: C, 81.41; H, 10.77; N, 3.65. Found: C, 81.24; H, 10.54; N, 3.66. IR λ_{max}^{Nujol} cm^{-1}: 3200, 3050 (NH), 1670 (CONH), 1585 ($\Delta^{2,4}$). UV λ_{max}^{EtOH}: 297 mμ (ε 12,500).

(ii) Treatment of 300 mg. of the formate IIIa in 50 cc. of anhyd. benzene with dry NH_3 in the same manner as above afforded 60 mg. of IX of m.p. 185~187°, which was identical in all respects with the sample obtained as above.

(iii) A solution of 500 mg. of the acetate IVa in 50 cc. of anhyd. benzene was treated with dry NH_3 and worked up as described above. Silica gel-chromatography of the product and recrystallization from hexane gave 110 mg. of IX as needles melting at 186~188° (decomp.).

5-Oxo-5,7-seco-B-norcholest-3-en-7-oic Amide (XII)——A solution of 70 mg. of 3β-acetoxy-6-oxacholest-4-en-7-one (VIIIa) in 70 cc. of anhyd. benzene was treated with a stream of dry NH_3 for 3 hr., and the solution was kept for 24 hr. at room temperature. The solution was concentrated to a small volume, and the precipitates were collected by filtration, and recrystallized from hexane-benzene to give 5-oxo-5,7-seco-B-norcholest-3-en-7-oic amide (XII) as needles, m.p. 204° (decomp.), $[\alpha]_D^{28}$ +108° (c=1.417). *Anal.* Calcd. for $C_{26}H_{43}O_2N$: C, 77.75; H, 10.79; N, 3.49. Found: C, 77.59; H, 10.56; N, 3.15. IR λ_{max}^{Nujol} cm^{-1}: 3520, 3380, 3260 (NH), 1680 (5-CO), 1635 (-$CONH_2$). UV λ_{max}^{EtOH} 226 mμ (ε 8,600).

Ozonolysis of 3β-Acetoxyandrost-5-en-7,17-dione (Ib)——A solution of 10 g. of Ib in a mixture of 450 cc. of AcOH and 450 cc. of AcOEt was treated with a steam of an ozonized air (0.11 mole of O_3/hr.) for 5 hr. A mixture of 10 cc. of 30% H_2O_2 and 20 cc. of water was added to the solution. After vigorous shaking the mixture was left standing overnight at room temperature. The solvent was removed under reduced pressure below 50° to give a residue, which was dissolved in AcOEt and fractionated with 5% $NaHCO_3$ solution into an acidic and a neutral substance.

The organic layer was washed well with water until neutral, dried, and the solvent evaporated to afford 4.1 g. of 3β-acetoxy-5-formoxy-6-oxa-5ξ-androstan-7,17-dione (IIIb), which after recrystallization from hexane-benzene formed needles, m.p. 205° (decomp.), $[\alpha]_D^{31}$ +21° (c=1.53). *Anal.* Calcd. for $C_{21}H_{28}O_7$: C, 64.27; H, 7.19. Found: C, 64.62; H, 7.13. IR λ_{max}^{Nujol} cm^{-1}: 1757 (lactone CO), 1744 (17-CO), 1735 (AcO), 1727 (HCOO); $\lambda_{max}^{CCl_4}$ cm^{-1}: 1780~1770 (broad; lactone CO), 1748 (strong; CO).

The aq. alkaline layer was acidified with dil. HCl, extracted with AcOEt, and the extract washed with water until neutral, and dried. The solvent was evaporated to give 2.9 g. of a white crystalline product, which was chromatographed over 90 g. of silica gel. The eluate with $CHCl_3$ containing 2% EtOH gave 2.0 g. of 3β-acetoxy-5,17-dioxo-5,7-seco-B-norandrostan-7-oic acid (IIb), which after recrystallization from hexane-benzene showed m.p. 177° (decomp.) and $[\alpha]_D^{28}$ +153° (c=1.886). *Anal.* Calcd. for $C_{20}H_{28}O_6 \cdot H_2O$: C, 62.81; H, 7.91. Found: C, 62.54; H, 7.99.

The methyl ester of IIb prepared by the action of CH_2N_2 on the acid IIb, showed m.p. 194~195° and $[\alpha]_D^{31}$ +145° (c=1.630), after recrystallization from hexane-benzene. *Anal.* Calcd. for $C_{21}H_{30}O_6$: C, 66.64; H, 7.99. Found: C, 66.86; H, 8.16. On chromatography over neutral alumina (Woelm; grade II) the methyl ester was partly converted into methyl 5,17-dioxo-5,7-seco-B-norandrost-3-en-7-oate (cf. Vb), m.p. 101°, $[\alpha]_D^{31}$ +262° (c=1.757). *Anal.* Calcd. for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found: C, 71.38; H, 8.02. UV: λ_{max}^{EtOH} 226.5 mμ (ε 8,300).

3β,5-Diacetoxy-6-oxa-5ξ-androstan-7,17-dione (IVb)——A solution of 500 mg. of 3β-acetoxy-5,17-dioxo-5,7-seco-B-norandrostan-7-oic acid (IIb) in 5 cc. of Ac_2O was heated at 80~85° for 2 hr. The solvent was removed under reduced pressure to give a syrupy substance, which was chromatographed over 15 g. of silica gel. Elution with benzene-$CHCl_3$ (1 : 1) gave 410 mg. of 3β,5-diacetoxy-6-oxa-5ξ-androstan-7,17-dione (IVb), which after recrystallization from hexane-benzene had m.p. 185°, $[\alpha]_D^{27}$ +92.4° (c=2.382). *Anal.* Calcd. for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 64.91; H, 7.40. IR λ_{max}^{Nujol} cm^{-1}: 1761, 1756 (lactone CO), 1743 (17-CO), 1740 (AcO); $\lambda_{max}^{CCl_4}$ cm^{-1}: 1776 (lactone CO), 1748 (strong; CO).

5,17-Dioxo-5,7-seco-B-norandrost-3-en-7-oic Acid (Vb)——(i) A solution 100 mg. of 3β-acetoxy-5,17-dioxo-5,7-seco-B-norandrostan-7-oic acid (IIb) in 10 cc. of 0.5*N* NaOH solution was heated at 70~80° on a steam-bath for 30 min. The mixture was neutralized with dil. HCl, extracted with AcOEt, the extract washed with water until neutral, dried, and the solvent was evaporated. The residue after recrystallization from hexane-Et_2O yielded 60 mg. of 5,17-dioxo-5,7-seco-B-norandrost-3-en-7-oic acid (Vb), m.p. 167~169°. *Anal.* Calcd. for $C_{18}H_{24}O_4$: C, 71.02; H, 7.95. Found: C, 71.11; H, 7.96. IR λ_{max}^{Nujol} cm^{-1}: 1730 (17-CO), 1702 (COOH), 1670 (5-CO).

(ii) Hydrolysis of 3β-acetoxy-5-formoxy-6-oxa-5ξ-androstan-7,17-dione (IIIb) with 0.5*N* NaOH solution was accomplished in the same manner as described above. The product was purified by recrystallization from hexane-ether to give Vb of m.p. 166~168°, which was identical with the sample obtained above.

6-Oxaandrosta-2,4-dien-7,17-dione (VIIb)——(i) A solution of 500 mg. of 3β-acetoxy-5-oxo-5,7-seco-B-norandrostan-7-oic acid (IIb) in 4 cc. of Ac_2O and 2 cc. of AcCl was refluxed under a N_2 atmosphere for 28 hr. The solvent was removed under reduced pressure below 40° to give only substance, which was chromatographed over 15 g. of silica gel. The benzene eluate yielded 350 mg. of 6-oxaandrosta-2,4-dien-7,17-dione (VIIb), m.p. 195~200°, raised to 202~204°, and $[\alpha]_D^{28}$ +145° (c=1.01). After recrystallization from hexane-benzene. *Anal.* Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74. Found: C, 75.61; H, 7.93. IR λ_{max}^{Nujol} cm^{-1}: 1740 (17-CO, lactone CO), 1655, 1585 ($\Delta^{2,4}$). UV: λ_{max}^{EtOH} 273 mμ (ε 8.700).

(ii) A solution of 500 mg. of IIb and 10 mg. of freshly fused AcONa in 10 cc. of Ac_2O was refluxed under N_2 for 2 hr. The mixture was concentrated to a small volume under reduced pressure, and treated with water and AcOEt. The organic layer was washed well with water until neutral, dried, and the solvent evaporated to afford a solid residue, which was chromatographed over 15 g. of silica gel. The first fraction eluted with benzene gave 250 mg. of VIIb of m.p. 201~202° (from hexane-benzene), which was proved to be identical with the sample obtained above.

The second fraction eluted with benzene-$CHCl_3$ (1 : 1) gave 69 mg. of the enol lactone VIIIb, m.p. 195~196°, $[\alpha]_D^{29}$ +63° (c=1.903). *Anal.* Calcd. for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.39; H, 7.46. IR λ_{max}^{Nujol} cm^{-1}: 1735 (broad; 17-CO, AcO, lactone CO), 1670 (Δ^4).

Treatment of 3β-Acetoxy-5,17-dioxo-5,7-seco-B-norandrostan-7-oic Acid (IIb) with Acetic-formic Anhydride——A solution of 300 mg. of IIb in 4 cc. of acetic-formic anhydride was refluxed at 70~80° for 20 min. To the cooled solution 30 cc. of AcOEt and 20 cc. of water were added and the mixture was set aside for 2 hr. at room temperature. The organic layer was washed successively with 2% $NaHCO_3$ solution and water, until neutral, dried, and the solvent evaporated under reduced pressure. A syrupy residue was chromatographed over 10 g. of silica gel. The eluate with benzene gave 90 mg. of 6-oxaandrosta-2,4-dien-7,17-dione (VIIb), which after recrystallization from hexane-benzene melted at 202~204°. The fraction eluted with benzene-$CHCl_3$ (3 : 2) yielded 13 mg. of 3β-acetoxy-6-oxaandrost-4-en-7,17-dione (VIIIb), m.p. 195°.

The third fraction eluted with $CHCl_3$ gave 7 mg. of 3β,5-diacetoxy-6-oxa-5ξ-androstan-7,17-dione (IVb), m.p. 182~184°. The last fraction with the same solvent afforded 50 mg. of 3β-acetoxy-5-formoxy-6-oxa-5ξ-androstan-7,17-dione (IIIb), m.p. 202~204°. The identity of all the four substances obtained in this experiment was confirmed by mixed m.p. determination and IR spectra comparison with the respective compounds obtained earlier.

3β-Acetoxy-5-hydroxy-6-aza-5ξ-androstan-7,17-dione (Xb)——(i) A solution of 500 mg. of 3β-acetoxy-5-formoxy-6-oxa-5ξ-androstan-7,17-dione (IIIb) in 150 cc. of dry benzene was bubbled with a stream of dry NH_3 for 3 hr. and allowed to stand overnight at room temperature. The solvent was removed under reduced pressure at 40~45° to give a white crystalline product. Recrystallization from hexane-benzene yielded 355 mg. of 3β-acetoxy-5-hydroxy-6-aza-5ξ-androstane-7,17-dione (Xb) as needles, m.p. 146° (decomp.). It was unstable in the air, and became a brown syrupy substance within a day at room temperature. *Anal.* Calcd. for $C_{20}H_{29}O_5N$: C, 66.09; H, 8.04; N, 3.85. Found: C, 66.14; H, 8.28; N, 8.69. IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3700 (OH), 3400 (NH), 1730 (17-CO, AcO), 1650 (CONH); λ_{max}^{Nujol} cm^{-1}: 3425 (OH, NH), 1730 (17-CO), 1715 (AcO), 1645, 1625 (CONH).

(ii) A solution of 1.5 g. of 3β,5-diacetoxy-6-oxa-5ξ-androstan-7,17-dione (IVb) in 200 cc. of dry benzene was treated with dry NH_3 for 4 hr., and kept for 2 days at room temperature. After evaporation of the solvent under reduced pressure below 45°, the residue was chromatographed over 50 g. of neutral alumina (Woelm; grade III). The eluate with $CHCl_3$ afforded 1.3 g. of (Xb), m.p. 144° (decomp.), which showed no depression of m.p. on admixture with the sample obtained above.

5-Hydroxy-6-aza-5ξ-androst-2 or 3-en-7,17-dione (XI)——A solution of 200 mg. of 6-oxaandrosta-2,4-dien-7,17-dione (VIIb) in 50 cc. of dry benzene was treated with dry NH_3 for 2 hr. at room temperature and left standing for 2 days. Upon concentrating the solvent below 50° under reduced pressure, there were obtained 123 mg. of XI of m.p. 214~215°. Recrystallization from hexane-$CHCl_3$ yielded silky needles, m.p. 230°, $[\alpha]_D^{29}$ +43° (c=1.366). *Anal.* Calcd. for $C_{18}H_{25}O_3N$: C, 71.25; H, 8.31; N, 4.62. Found: C, 71.12; H, 8.11; N, 4.95. IR λ_{max}^{Nujol} cm^{-1}: 3470 (OH, NH), 1740 (17-CO), 1647, 1623 (CONH).

Ozonolysis of 3β-Acetoxypregn-5-en-7,20-dione (Ic)——A solution of 5 g. of Ic in a mixture of 250 cc. of AcOEt and 250 cc. of AcOH was treated with an ozonized-air at 0°. To the reaction mixture 20 cc. of 3% H_2O_2 was added and the solution kept overnight at room temperature. The organic layer was washed with cold 1% NaOH solution, then water until neutral, dried, and the solvent removed to give 4.38 g. of an amorphous material, which on chromatography over neutral alumina (Woelm; grade V) using benzene as a solvent afforded 2.5 g. of 3β-acetoxy-5-formoxy-6-oxa-5ξ-pregnan-7,20-dione (IIIc). Recrystallization from hexane-benzene gave plates, m.p. 152~153° (decomp.). *Anal.* Calcd. for $C_{23}H_{32}O_7$: C, 65.69; H, 7.67. Found: C, 65.73; H, 7.61. IR λ_{max}^{Nujol} cm^{-1}: 1765 (lactone CO), 1743 (AcO, HCOO), 1706 (20-CO); $\lambda_{max}^{CCl_4}$ cm^{-1}: 1774 (lactone CO), 1743 (AcO, HCOO), 1717 (20-CO).

The aq. alkaline layer was acidified with AcOH and extracted with Et_2O. The extract was worked up as usual and yielded 2.1 g. of a syrupy acid IIc, which did not crystalize.

6-Oxapregna-2,4-diene-7,20-dione (VIIc)——Three grams of the oily acid IIc obtained above was dissolved in 25 cc. of Ac_2O containing 25 mg. of freshly fused AcONa and the mixture was refluxed

for 2 hr. under a N_2 atmosphere. The solvent was removed under reduced pressure, water added, and the product was extracted with Et_2O. The extract was washed with 2% $NaHCO_3$ solution, dried, and the solvent removed to give 2.63 g. of an oily residue, which was chromatographed on neutral alumina (Woelm; grade III). The benzene fraction gave 743 mg. of 6-oxapregna-2,4-diene-7,20-dione (VIIc), which on recrystallization from hexane-benzene gave needles, m.p. 157~159°. *Anal.* Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 75.98; H, 8.56. IR λ_{max}^{KBr} cm^{-1}: 1750 (lactone CO), 1710 (20-CO), 1650, 1590 ($\Delta^{2,4}$). UV: λ_{max}^{EtOH} 273 mμ (ε 8,400).

3β-Acetoxy-5-hydroxy-6-aza-5ξ-pregnan-7-one (Xc)——A solution of 500 mg. of the formate IIIc in 150 cc. of dry benzene was bubbled with dry NH_3 for 2.5 hr., and left standing overnight at room temperature. The solvent was removed to give an amorphous substance, which on repeated recrystallization from hexane-benzene afforded 3β-acetoxy-5-hydroxy-6-aza-5ξ-pregnan-7-one (Xc) as fine needles, m.p. 127~127.5° (decomp.). *Anal.* Calcd. for $C_{22}H_{33}O_5N$: C, 67.49; H, 8.50; N, 3.58. Found: C, 67.26; H, 8.32; N, 3.61. IR λ_{max}^{KBr} cm^{-1}: 3450 (OH), 3230, 3100 (NH), 1725 (AcO), 1708 (20-CO), 1655 (CONH).

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Summary

Ozonolysis of 3β-acetoxycholest-5-en-7-one (Ia), 3β-acetoxyandrost-5-en-7,17-dione (Ib), and 3β-acetoxypregn-5-en-7,20-dione (Ic) afforded 3β-acetoxy-5-formoxy-6-oxa-5ξ-cholestan-7-one (IIIa), 3β-acetoxy-5-formoxy-6-oxa-5ξ-androstan-7,17-dione (IIIb), and 3β-acetoxy-5-formoxy-6-oxa-5ξ-pregnan-7,20-dione (IIIc), respectively. The corresponding δ-keto acids (IIa, b, c) were also isolated from the reaction mixture. Some 6-oxa- and 6-aza-steroids were derived from the δ-keto acid II as well as from its pseudo acid formate III and acetate IV.

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40. Tanezo Taguchi, Keiro Yoshizue,[*1] and Shiro Anzai[*2]: Configurational Effect in N-Alkylation of Diastereomeric 2-Aminocyclohexanol.[*3]

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Some years ago Taguchi and Nakayama[1] reported N-ethylation of DL-*trans*-and DL-*cis*-2-aminocyclohexanols (I) with ethyl tosylate or ethyl bromide and potassium carbo nate, in which *trans*-I underwent N-diethylation, whereas *cis*-I gave an N-monoethyl derivative, which resisted further ethylation. This phenomenon was considered due to a hydrogen bond,[2~4] $-\overset{|}{\underset{|}{N}}\cdots H-O-$, which exists in the N-ethyl derivative of *cis*-I,

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4) The hydrogen bond was erroneously drawn between O and H attached to N in the diagram of the N-ethyl derivative of *cis*-I in the preceding paper.[1]