Treatment of IVc in pyridine with acetic anhydride in the usual manner gave IVd which was unable to crystallize. NMR (5% solution in $CDCl_3$) δ: 0.89 (6H, s, 18- and 19-CH_3), 2.03, 2.06 (3H, 3H, s, s, 3-CH_2OCOCH_3 and 5α-$OCOCH_3$), 4.00 (2H, m, 3-CH_2OCOCH_3), 4.74 (1H, m, $W1/2=6$ Hz, 5β-H), 4.90 (2H, m, 21-CH_2), 5.90 (1H, m, 22-H).

3-Oxo-4-oxa-14-hydroxy-5α,14β-card-20(22)-enolide (Va) and 3-Oxo-4-oxa-14-hydroxy-5β,14β-card-20(22)-enolide (Vb)——To a solution of IIIa (83 mg) in a mixture of methanol (12 ml) and water (1 ml) was added portionwise $NaBH_4$ (100 mg) under stirring for 2 hr at 0°. The reaction mixture was poured into ice-water, acidified to pH 4 with 2 N HCl under continuous stirring for 1 hr at 0°. After neutralization of the reaction mixture with 5% $NaHCO_3$ the resulting solution was concentrated *in vacuo* to a small volume, and then extracted with chloroform. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded a crystalline residue which was recrystallized from acetone–hexane to afford Va (24 mg) as colorless leaflets. mp 275—282°. *Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.70; H, 8.08. IR ν_{max} cm^{-1}: 3520 (OH), 1795, 1735, 1635 (butenolide and δ-lactone). NMR (5% solution in $CDCl_3$) δ: 0.91 (sh), 0.92 (6H, s, 18- and 19-CH_3), 3.98 (1H, m, $W1/2=19$ Hz, 5α-H), 4.92 (2H, m, 21-CH_2), 5.91 (1H, m, 22-H).

The mother liquors of recrystallization of Va were combined and evaporated *in vacuo* to give a crystalline residue (52 mg) which was submitted to preparative TLC using chloroform–methanol (49: 1) as solvent. A fraction corresponding to the spot (*Rf*: 0.41; *Rf* of Va: 0.38) afforded Vb (26 mg) as colorless leaflets after recrystallization from acetone–hexane. mp 265—271°. *Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.71; H, 8.05. IR ν_{max} cm^{-1}: 3500 (OH), 1795, 1760, 1720, 1640 (butenolide and δ-lactone). NMR (5% solution in $CDCl_3$) δ: 0.90, 1.00 (3H, 3H, s, s, 18- and 19-CH_3), 4.17 (1H, m, $W1/2=5$ Hz, 5β-H), 4.89 (2H, m, 21-CH_2), 5.88 (1H, m, 22-H).

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Synthesis of Epimeric 6-Deuterio-4-cholesten-3-ones[1]

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In order to clarify the stereochemistry of hydrogen transfer from C-4 to C-6 during enzymatic transformation of cholesterol into cholestenone, the title compounds stereospecifically labeled with deuterium have been synthesized as an authentic specimen. The stereospecific labeling at 6β was attained by *trans*-diaxial opening of the 5α,6α-oxido ring with lithium aluminum deuteride. Oxidation with chromium trioxide followed by dehydration with *p*-toluenesulfonic acid provided 6β-d_1-cholestenone (**4a**). In the similar fashion 6α-d_1-cholestenone (**4b**) was prepared from 6β-d_1-3α,5-cyclo-5α-cholestan-6α-ol (**6**) by way of 6-d_1-cholesteryl acetate (**7**).

Keywords——$\Delta^5 \rightarrow \Delta^4$ isomerization; cholesterol oxidase; stereospecific labeling; lithium aluminum deuteride; *trans*-diaxial opening of epoxide; 6-d_1-5α-hydroxycholestan-3-ones; 6-d_1-5α-cholestane-3β,5-diols

It has previously been demonstrated that cholesterol oxidase from *Brevibacterium sterolicum* is capable of transforming cholesterol into 4-cholesten-3-one.[3] Although the properties of this enzyme have been disclosed to a certain extent,[4] the steric mechanism of migration

1) Part CXXV of "Studies on Steroids" by T. Nambara; Part CXXIV: H. Hosoda, K. Yamashita, K. Tadano, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **25**, 2650 (1977).
2) Location: *Aobayam, Sendai.*
3) T. Uwajima, H. Yagi, and O. Terada, *Agr. Biol. Chem.* (Tokyo), **37**, 2345 (1973).
4) T. Uwajima, H. Yagi, and O. Terada, *Agr. Biol. Chem.* (Tokyo), **38**, 1149 (1974).

of the double bond still remains unclear. As a series of our studies on the biotransformation of steroids,[5] elucidation of the stereochemistry of proton transfer from C-4 to C-6 during enzymatic isomerization has been undertaken. The design of the experiment required cholestenone stereospecifically labeled with deuterium at C-6 as an authentic sample. The methods so far available for introducing deuterium into the C-6 position in cholestenone are not yet satisfactory in respect of the stereospecificity and yield.[6] The present paper deals with the convenient synthesis of epimeric 6-deuteriocholestenones starting from cholesterol.

An initial project was directed to deuterium labeling at the 6β position by *trans*-diaxial opening of the 5α,6α-epoxide. Reductive cleavage of 5,6α-epoxy-5α-cholestan-3β-ol (**1**), readily obtainable from cholesterol, with lithium aluminum deuteride afforded the 6β-deuterated 3β,5α-diol (**2a**), which on oxidation with chromium trioxide was led to the 3-keto derivative (**3a**). Subsequent dehydration was effected by treatment with *p*-toluenesulfonic acid to provide the desired 6β-d_1-cholestenone (**4a**) in a fairly good yield.

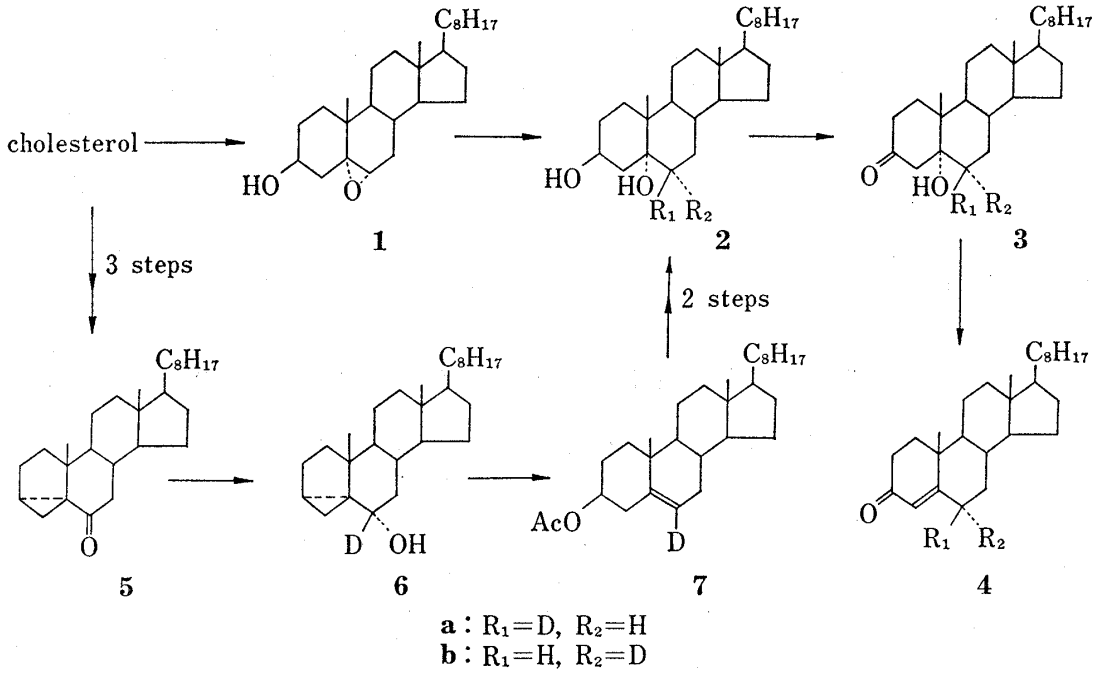

Chart 1

The preparation of the epimeric 6α-deuterated compound was then carried out employing 6-d_1-cholesteryl acetate (**7**) as a key intermediate. Treatment of 3α,5-cyclo-5α-cholestan-6-one (**5**), derivable from cholesterol in three steps, with lithium aluminum deuteride furnished 6β-deuterated 6α-hydroxy compound (**6**) as a sole product. When **6** was exposed to acetic acid in dry ether in the presence of boron trifluoride etherate, regeneration of the 5,6-double bond took place yielding the desired Δ^5-3β-ol acetate (**7**). Disappearance of the signal due to the C-6 proton in the nuclear magnetic resonance (NMR) spectrum justified the retention of the heavy isotope at C-6. Transformation of **7** into the Δ^4-3-ketone was undertaken in the similar fashion as described above. Being treated with per acid, **7** was converted to the

5) T. Anjyo, M. Ito, H. Hosoda, and T. Nambara, *Chem. Ind.* (London), **1972**, 384; T. Nambara, T. Anjyo, M. Ito, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 1938 (1973); S. Ikegawa and T. Nambara, *Chem. Ind.* (London), **1973**, 230; T. Nambara, S. Ikegawa, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 2794 (1973); T. Nambara, S. Ikegawa, and M. Kato, *ibid.*, **23**, 2164 (1975); T. Nambara, S. Ikegawa, and C. Takahashi, *ibid.*, **23**, 2358 (1975); T. Nambara, S. Ikegawa, H. Ishida, and H. Hosoda, *ibid.*, "in press."

6) J.C. Jacquesy, R. Jacquesy, and J. Levisalles, *Bull. Soc. Chim. Fr.*, **1967**, 1649.

5α,6α-epoxide which in turn was submitted to further elaboration without purification. Reductive cleavage with lithium aluminum hydride followed by chromatographic separation on silica gel furnished the 6α-deuterio-3β,5α-dihydroxy compound (**2b**). Subsequent oxidation with chromium trioxide gave the 3-ketone (**3b**) which on dehydration with *p*-toluenesulfonic acid under mild conditions was led to the desired 6α-d_1-cholestenone (**4b**).

The infrared (IR) spectra of non-labeled and epimeric 6-deuterated cholestenones were obviously distinguishable one another in the finger print region. Inspection of the molecular ion peak in the mass spectra (MS) revealed that the isotopic purity of these deuterated compounds was more than 98%. In the NMR spectra the C-4 proton signal of these labeled compounds (**4a**, **4b**) appeared at 5.70 ppm as a sharpened singlet with a line width of 2 Hz and at 5.68 ppm as a doublet (J=1.5 Hz) supporting the retention of the label at the 6β and 6α position, respectively.

The results on the stereochemistry of enzymatic isomerization by cholesterol oxidase will be the subject of a future communication.

Experimental[7]

6β-d_1-5α-Cholestane-3β,5-diol (**2a**)——To a solution of 5,6α-epoxy-5α-cholestan-3β-ol (**1**) (450 mg) in anhydrous tetrahydrofuran (THF) (10 ml) was added $LiAlD_4$ (300 mg) and refluxed for 2.5 hr. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from MeOH gave **2a** (305 mg) as colorless leaflets. mp 218—219°.

6β-d_1-5α-Hydroxycholestan-3-one (**3a**)——To a stirred solution of **2a** (130 mg) in pyridine (4 ml) was added CrO_3–pyridine complex (1: 10 w/v) (5 ml) and allowed to stand at room temperature for 5 hr. The reaction mixture was diluted with AcOEt, washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, succesively and dried over anhydrous Na_2SO_4. After usual work-up recrystallization of the crude product from acetone gave **3a** (113 mg) as colorless leaflets. mp 227—228°.

6β-d_1-Cholest-4-en-3-one (**4a**)——To a solution of **3a** (17 mg) in benzene (5 ml) was added anhydrous *p*-toluenesulfonic acid (8 mg) and stirred at room temperature for 42 hr. The reaction mixture was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC using hexane–AcOEt (6: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **4a** (10 mg) as colorless plates. IR ν_{max}^{KBr} cm^{-1}: 2140 (C–D). mp 80.5—81°. NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.87 (6H, d, J=6 Hz, 26- and 27-CH_3), 0.92 (3H, d, J=6 Hz, 21-CH_3), 1.17 (3H, s, 19-CH_3), 5.70 (1H, s, C_4–H). MS *m/e*: 385 (M^+) (98% d_1).

6-d_1-Cholest-5-en-3β-ol Acetate (**7**)——To a solution of 3α,5-cyclo-5α-cholestan-6-one (**5**) (1 g) in anhydrous THF (10 ml) was added $LiAlD_4$ (300 mg) and refluxed for 1.5 hr. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. To a solution of this residue in anhydrous ether (50 ml) were added AcOH (15 ml) and BF_3-etherate (15 ml) under ice-cooling, and stirred at 0° for 1.5 hr. The reaction mixture was diluted with ether, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product (1.1 g) was submitted to further elaboration without purification. A portion of the crude product was recrystallized from MeOH to give **7** as colorless leaflets. mp 114—115°. NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.87 (6H, d, J=6 Hz, 26- and 27-CH_3), 0.92 (3H, d, J=6 Hz, 21-CH_3), 1.03 (3H, s, 19-CH_3), 2.05 (3H, s, –$OCOCH_3$), 4.64 (1H, m, 3α-H).

6α-d_1-5α-Cholestane-3β,5-diol (**2b**)——To a solution of **7** (1 g) in benzene (20 ml) was added *m*-chloroperbenzoic acid (400 mg) and stirred at room temperature overnight. The reaction mixture was diluted with AcOEt, washed with 5% $NaHSO_3$, 5% $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, and evaporated. To a solution of the crude product in anhydrous THF (10 ml) was added $LiAlH_4$ (300 mg) and refluxed for

7) All melting points were taken on a micro hot-stage apparatus and are uncorrected. IR spectra were obtained on a JASCO Model IRA-1 spectrometer. NMR spectra were recorded on a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet. Mass spectra were measured by a Hitachi Model RMU-7 spectrometer. For preparative thin-layer chromatography (TLC) Silica gel H and Silica gel HF_{254} (E. Merck AG, Darmstadt) were used as an adsorbent. Isotopic purity of $LiAlD_4$ used was over 98%. Mixed melting point of the deuterated compounds on admixture with the non-labeled anthentic samples showed no depression, respectively.

2 hr. After addition of moist AcOEt to decompose the excess reagent the resulting solution was diluted with 20% Rochelle salt solution and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel. Elution with hexane–AcOEt (5: 1) and recrystallization of the eluate from MeOH gave **2b** (424 mg) as colorless leaflets. mp 218—219°.

6α-d_1-5α-Hydroxycholestan-3-one (**3b**)——Oxidation of **2b** (120 mg) with CrO_3-pyridine complex (1: 10 w/v) was carried out in the manner as described with **2a**. Recrystallization of the crude product gave **3b** (92 mg) as colorless leaflets. mp 227—228°.

6α-d_1-Cholest-4-en-3-one (**4b**)——Dehydration of **3b** (93 mg) with anhydrous *p*-toluenesulfonic acid (50 mg) in benzene (15 ml) was carried out in the manner as described with **3a**. The crude product was purified by preparative TLC using hexane–AcOEt (6: 1) as developing solvent. Recrystallization of the eluate from MeOH gave **4b** (58 mg) as colorless plates. IR ν_{max}^{KBr} cm^{-1}: 2150 (C–D). mp 80.5—81°. NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.86 (6H, d, J=6 Hz, 26- and 27-CH_3), 0.90 (3H, d, J=6 Hz, 21-CH_3), 1.18 (3H, s, 19-CH_3), 5.68 (1H, d, J=1.5 Hz, C_4–H). MS *m/e*: 385 (M^+) (98% d_1).

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Studies on Peptides. LXXII.[1,2] Examination of the N^{ε}-Alkylation of Lysine in the Methanesulphonic Acid Procedure for Peptide Synthesis

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Methanesulphonic acid cleaved the Z group from H–Lys(Z)–OH without concomitant formation of H–Lys(Bzl)–OH in the presence of the cation scavenger, anisole.

Keywords——N^{ε}-benzyllysine; alkylation of N^{ε}–Lys in the trifluoromethanesulphonic acid–trifluoroacetic acid–anisole system; deprotection in the trifluoromethanesulphonic acid–trifluoroacetic acid–thioanisole system; deprotection in the methanesulphonic acid–anisole system; examination of cation scavengers; intramolecular alkylation

In 1975, we reported that methanesulphonic acid (MSA) cleaved the most of protecting groups currently employed in peptide synthesis.[4] However, regeneration of Arg from Arg (NO_2) or Arg (Tos) with this reagent was not effective enough compared to the results of trifluoromethanesulphonic acid (TFMSA).[5] We had therefore to apply the latter stronger acid as a deprotecting reagent at the final step of the synthesis of peptides containing Arg,

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2) Amino acids mentioned here are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Bzl=benzyl, TFA=trifluoroacetic acid.
3) Location: *Sakyo-ku, Kyoto, 606, Japan.*
4) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 (1975).
5) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J.C.S. Chem. Commun.*, **1974**, 107.