toluene for **2b**—**f** and with $CHCl_3$ for **3**. The product, if an oil, was converted into the maleate or hydrochloride; if solid, it was purified by recrystallization. The results are summarized in Tables I and II.

5-Benzyl-7-methyl-1,2,3,4-tetrahydropyrimido[1,6-*a*]indole (4)——A mixture of **2e** (15 g) and 5% Pd-C (3 g) in 70% EtOH (370 ml) was subjected to catalytic hydrogenation at 60° under normal pressure. After the theoretical amount of H_2 had been absorbed, the catalyst was removed and 70% of the solvent was removed *in vacuo*. The precipitated crystals were collected and recrystallized from dil. EtOH to give 6.6 g (51.6%) of **4**.

5-Benzyl-2-[3-(*p*-fluorobenzoyl)propyl]-1,2,3,4-tetrahydropyrimido[1,6-*a*]indoles (5)——The benzylation of **6** with benzyl chloride was carried out by the procedure described for the alkylation of **1** using xylene as a solvent. The crude product was chromatographed on silica gel, and elution with toluene–$CHCl_3$ (20 : 1) gave **5** as an oily product.

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Synthesis of Conjugated Cholesterol and Cholestanols[1]

JUNICHI GOTO, KAZUHIKO SUZAKI,[2] and TOSHIO NAMBARA[2a]

Pharmaceutical Institute, Tohoku University[2]

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The glucuronides, sulfates, glucosides and N-acetylglucosaminides of cholesterol and epimeric 5α-cholestan-3-ols have been synthesized. The formation of a β-glucoside linkage was readily achieved by means of the Koenigs-Knorr reaction with the corresponding α-acetohalosugar, employing cadmium carbonate as a catalyst. The preparation of 6,7α,7β-d_3-cholesterol glucuronide is also described.

Keywords——cholesterol; 5α-cholestan-3β-ol; 5α-cholestan-3α-ol; Koenigs-Knorr reaction; cadmium carbonate; glucuronide; glucoside; N-acetylglucosaminide; sulfate; d_3-cholesterol glucuronide

It is reasonably well substantiated that cholesterol sulfate is an activated precursor in the biosynthesis of steroid hormones.[3,4] In 1970, Wade reported the occurrence of cholesterol glucuronide in human blood,[5] but the metabolic and physiological significance of this conjugate still remains unclear. Conjugation of cholesterol appears to be an important biotransformation in living animals in connection with the biosynthesis of bile acids as well as steroid hormones. In recent years, considerable attention has been focused on the marked elevation of the plasma level of 5α-cholestan-3β-ol in patients with cerebrotendinous xanthomatosis.[6–8] On the other hand novel conjugated forms other than the common glucuronide

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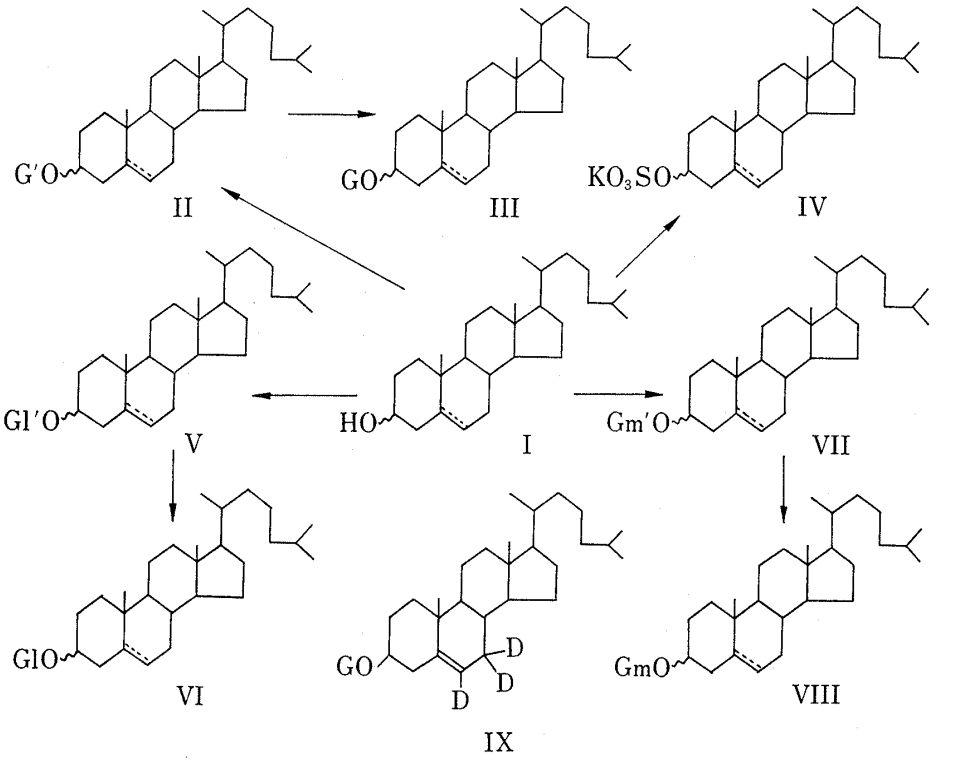

a : 3α-H, Δ^5 b : 3α-H, 5α-H c : 3β-H, 5α-H

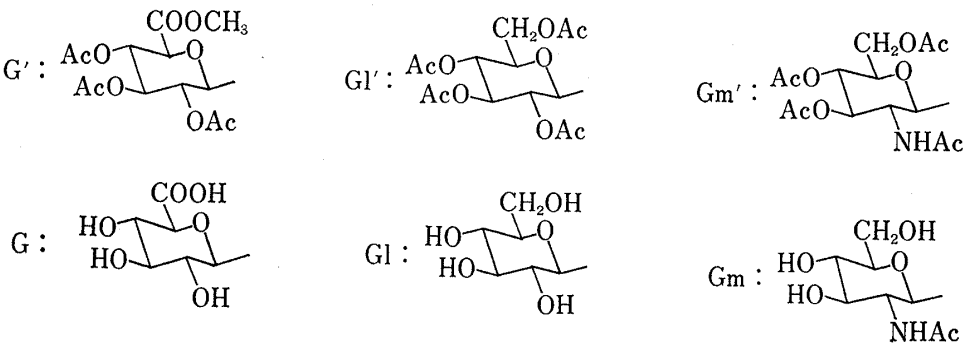

Chart 1

and sulfate have recently been demonstrated in the metabolism of steroid hormones.[9–12] These findings prompted us to prepare the glucuronides, sulfates, glucosides, and N-acetyl-glucosaminides of cholesterol and C-3 epimeric 5α-cholestan-3-ols.

The synthesis of steroid β-glucosides is usually attained by means of the Koenigs-Knorr reaction with an α-acetohalosugar in the presence of a catalyst. Since the first report by Bernstein and his co-worker,[13] cadmium carbonate has been widely used as a suitable catalyst for this purpose.[14,15] Koenigs-Knorr condensation of cholesterol (Ia) with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosiduronate in the presence of cadmium carbonate in anhydrous toluene provided cholesterol glucuronide acetate-methyl ester (IIa) in satisfactory yield. In a similar fashion, condensation of epimeric 5α-cholestan-3-ols (Ib, Ic) with an

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α-acetobromosugar afforded cholestanol glucuronide derivatives (IIb, IIc). Elimination of the protecting groups was effected by treatment with alkali in aqueous dioxane under mild conditions, yielding the desired β-glucuronides of cholesterol and epimeric 5α-cholestan-3-ols (IIIa, IIIb, IIIc).

The glucoside tetraacetates (Va, Vb, Vc) and N-acetylglucosaminide derivatives (VIIa, VIIb, VIIc) of the three steroids were similarly prepared by means of the Koenigs-Knorr reaction using 1-bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose and 1-chloro-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-α-D-glucopyranose, respectively. Subsequent removal of the protecting groups in V and VII furnished the desired β-glucosides (VIa, VIb, VIc) and N-acetyl-β-glucosaminides (VIIIa, VIIIb, VIIIc) in reasonable yields. The nuclear magnetic resonance (NMR) spectra of II, V, and VII weer indicative of the formation of the β-glucopyranoside linkage. The anomeric proton in the sugar moiety appeared near δ 4—5 as a doublet ($J=7$ Hz), indicating a *trans*-diaxial relationship to the vicinal 2′-proton.

The preparation of sulfates was readily attained by the usual method. On treatment with chlorosulfonic acid in pyridine, cholesterol and epimeric cholestanols were transformed into the desired sulfates (IVa, IVb, IVc) in excellent yields.

A particular interest in the metabolic significance of cholesterol glucuronide prompted us to prepare the deuterium-labeled compound for use in gas chromatography–mass spectrometry as an internal standard. After Koenigs-Knorr reaction followed by alkaline hydrolysis as described above, 6,7α,7β-d_3-cholesterol, which was readily obtainable by a known method, was converted to the desired glucuronide (IX). The isotopic purity of IX was determined by gas chromatography–mass spectrometry. The glucuronide was derivatized with diazomethane to give the methyl ester which in turn yielded the trimethylsilyl ether on treatment with trimethylsilyl imidazole in pyridine. The fragment ion at *m/e* 777 (M-15) resulting from loss of the methyl group justified the structural assignment of the derivative. Inspection of the base peak (*m/e* 369) which was formed by elimination of the sugar moiety, confirmed that the isotopic purity of deuterated cholesterol glucuronide was more than 87%.

The biological aspects of metabolic conjugation of cholesterol and related compounds seem to represent a fertile field. The availability of authentic steroid conjugates may be helpful for such studies.

Experimental[16]

General Procedure for the Preparation of Glucuronides

Freshly prepared $CdCO_3$ (860 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (500 mg) in anhydrous toluene (4 ml) were added to a solution of steroid (500 mg) in anhydrous toluene (30 ml), and the solvent was azeotropically distilled with stirring. After 2.5 hr, additional portions of the acetobromosugar (200 mg) in anhydrous toluene (2 ml) and $CdCO_3$ (300 mg) were added and the mixture was refluxed for another 2 hr. The precipitate was removed by filtration and washed with toluene. The filtrate and washings were combined and evaporated down *in vacuo*. The oily residue obtained was subjected to column chromatography on silica gel (20 g). Elution with hexane–AcOEt and recrystallization of the eluate from acetone–hexane gave the glucuronide acetate–methyl ester (IIa—c). KOH (12%, 3 ml) was added to a solution of II (70 mg) in dioxane (10 ml) and the resulting solution was stirred at room temperature overnight. The reaction mixture was acidified with 10% HCl and extracted with AcOEt. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated down. Recrystallization of the crude product from MeOH gave the glucuronide (IIIa—c).

General Procedure for the Preparation of Sulfates

Steroid (400 mg) in anhydrous pyridine (4 ml) was added to a solution of chlorosulfonic acid (0.4 ml)–anhydrous pyridine (4 ml) under ice-cooling and the mixture was stirred for 30 min at room temperature. The reaction mixture was poured into ice-water, and the precipitate was collected by filtration and washed with H_2O. Recrystallization of the crude product from MeOH gave the free sulfate. Methanolic KOH

16) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. NMR spectra were recorded on a JEOL PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard.

(10%, 1.5 ml) was added to a solution of the precipitate (20 mg) in MeOH (40 ml) and the mixture was evaporated down *in vacuo*. The crystalline material obtained was rinsed with H_2O and recrystallized from MeOH to give the sulfate (potassium salt) (IVa—c).

General Procedure for the Preparation of Glucosides

1-Bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (500 mg) in anhydrous toluene (4 ml) and freshly prepared $CdCO_3$ (500 mg) were added to a solution of steroid (500 mg) in anhydrous toluene (30 ml), and the solvent was azeotropically distilled with stirring. After 1.5 and 3 hr, additional portions of the acetobromosugar (300 mg) in anhydrous toluene (2 ml) and $CdCO_3$ (300 mg) were added and the mixture was refluxed for another 1.5 hr. The precipitate was removed by filtration and the filtrate was evaporated down *in vacuo*. The resulting pale yellow crystalline material was chromatographed on silica gel (20 g). Elution with hexane–AcOEt and recrystallization of the eluate from acetone–hexane gave the glucoside tetraacetate (Va—c). KOH (12%, 10 ml) was added to a solution of V (200 mg) in dioxane (30 ml) and the resulting solution was stirred at room temperature overnight. The reaction mixture was neutralized with 10% HCl and concentrated *in vacuo*. The residue was dissolved in H_2O and extracted with AcOEt–*n*-BuOH (3:1). The organic phase was dried over anhydrous Na_2SO_4 and evaporated down. Recrystallization of the crystalline material obtained from MeOH gave the glucoside (VIa—c).

General Procedure for the Preparation of N-Acetylglucosaminides

1-Chloro-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-α-D-glucopyranose (500 mg) in anhydrous benzene (8 ml) and freshly prepared $CdCO_3$ (860 mg) were added to a solution of steroid (500 mg) in anhydrous benzene (30 ml), and the solvent was azeotropically distilled with stirring. After 2 and 4 hr, additional portions of the acetochloroglucosamine (200 mg) in anhydrous benzene (4 ml) and $CdCO_3$ (200 mg) were added and the mixture was refluxed for another 2 hr. The precipitate was removed by filtration and the filtrate was concentrated *in vacuo*. The oily residue obtained was subjected to column chromatography on silica gel (20 g). Elution with hexane–AcOEt and recrystallization of the eluate from MeOH gave the N-acetylglucosaminide triacetate (VIIa—c). KOH (12%, 10 ml) was added to a solution of VII (200 mg) in dioxane (30 ml) and the resulting solution was stirred at room temperature overnight. The reaction mixture was neutralized with 10% HCl and extracted with AcOEt. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated down. Recrystallization of the crude product from MeOH gave the N-acetylglucosaminide (VIIIa—c).

Methyl (Cholest-5-en-3β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (II)——Yield 36%. mp 164—165°. $[\alpha]_D^{21}$ +10.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{62}O_{10}$: C, 68.35; H, 8.89. Found: C, 67.99; H, 8.83. NMR ($CDCl_3$) δ: 0.69 (3H, s, 18-CH_3), 0.88 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.93 (3H, d, J=7 Hz, 21-CH_3), 1.00 (3H, s, 19-CH_3), 2.06, 2.09 (9H, s, –OCOCH_3), 3.55 (1H, m, 3α-H), 3.80 (3H, s, –COOCH_3), 4.07 (1H, m, pyranose-C_5–H), 4.70 (1H, d, J=7 Hz, pyranose-C_1–H), 4.92—5.33 (3H, m, pyranose-C_2–, C_3– and C_4–H), 5.40 (1H, m, 5-H). Reported mp 176—178.[17]

Methyl (5α-Cholestan-3β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IIb)——Yield 51%. mp 180—181°. $[\alpha]_D^{21}$ −20.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15. Found: C, 68.33; H, 9.33. NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.86 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.93 (3H, d, J=7 Hz, 21-CH_3), 2.07, 2.09 (9H, s, –OCOCH_3), 3.55 (1H, m, 3α-H), 3.80 (3H, s, –COOCH_3), 4.10 (1H, m, pyranose-C_5–H), 4.70 (1H, d, J=7 Hz, pyranose-C_1–H), 4.96—5.37 (3H, m, pyranose-C_2–, C_3– and C_4–H).

Methyl (5α-Cholestan-3α-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IIc)——Yield 37%. mp 190—191°. $[\alpha]_D^{21}$ +10.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{64}O_{10}$: C, 68.15; H, 9.15. Found: C, 67.76; H, 9.25. NMR ($CDCl_3$) δ: 0.62 (3H, s, 18-CH_3), 0.76 (3H, s, 19-CH_3), 0.84 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.88 (3H, s, J=7 Hz, 21-CH_3), 2.02, 2.04 (9H, s, –OCOCH_3), 3.78 (3H, s, –COOCH_3), 3.98 (1H, m, 3β-H), 4.04 (1H, m, pyranose-C_5–H), 4.64 (1H, d, J=7 Hz, pyranose-C_1–H), 4.96—5.36 (3H, m, pyranose-C_2–, C_3– and C_4–H).

(Cholest-5-en-3β-yl-β-D-glucopyranosid)uronic Acid (IIIa)——Yield 59%. mp 225—228° (dec.). $[\alpha]_D^{21}$ −90.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{33}H_{54}O_7$: C, 70.43; H, 9.67. Found: C, 70.21; H, 9.92. NMR (DMSO-d_6) δ: 0.65 (3H, s, 18-CH_3), 0.85 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.96 (3H, s, 19-CH_3), 4.33 (1H, d, J=7 Hz, pyranose-C_1–H), 5.32 (1H, m, 5-H). Reported mp 242—245°.[17]

(5α-Cholestan-3β-yl-β-D-glucopyranosid)uronic Acid (IIIb)——Yield 59%. mp 210—212° (dec.). $[\alpha]_D^{21}$ +20.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{33}H_{56}O_7 \cdot 1/2H_2O$: C, 69.13; H, 9.93. Found: C, 69.16; H, 10.24. NMR (DMSO-d_6) δ: 0.62 (3H, s, 18-CH_3), 0.76 (3H, s, 19-CH_3), 0.84 (6H, d, J=7 Hz, 26- and 27-CH_3), 4.32 (1H, d, J=7 Hz, pyranose-C_1–H).

(5α-Cholestan-3α-yl-β-D-glucopyranosid)uronic Acid (IIIc)——Yield 69%. mp 207—210° (dec.). $[\alpha]_D^{21}$ +10.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{33}H_{56}O_7 \cdot 1/2H_2O$: C, 69.13; H, 9.9?. Found: C, 69.39; H, 10.33. NMR (DMSO-d_6) δ: 0.62 (3H, s, 18-CH_3), 0.73 (3H, s, 19-CH_3), 0.84 (6H, d, J=7 Hz, 26- and 27-CH_3), 4.24 (1H, d, J=7 Hz, pyranose-C_1–H).

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Cholest-5-en-3β-ol Sulfate Potassium Salt (IVa)——Yield 83%. mp 228°. $[\alpha]_D^{21}$ −30.0° (c=0.1, MeOH). Reported mp 220—225°.[17]

5α-Cholestan-3β-ol Sulfate Potassium Salt (IVb)——Yield 69%. mp 193°. $[\alpha]_D^{21}$ −10.0° (c=0.1, MeOH) Reported mp 234°.[18]

5α-Cholestan-3α-ol Sulfate Potassium Salt (IVc)——Yield 69%. mp 143—145°. $[\alpha]_D^{21}$ +60.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{27}H_{47}KO_4S \cdot H_2O$: C, 61.78; H, 9.41. Found: C, 61.91; H, 9.45. NMR (DMSO-d_6) δ: 0.60 (3H, s, 18-CH_3), 0.72 (3H, s, 19-CH_3), 0.82 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.86 (3H, d, J=7 Hz, 21-CH_3), 4.28 (1H, m, 3β-H).

Cholest-5-en-3β-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (Va)——Yield 24%. mp 163—164°. $[\alpha]_D^{21}$ −10.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{64}O_{10}$: C, 68.68; H, 9.00. Found: C, 68.97; H, 9.14. NMR ($CDCl_3$) δ: 0.74 (3H, s, 18-CH_3), 0.89 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.93 (3H, d, J=7 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 2.06, 2.08, 2.10, 2.12 (12H, s, -$OCOCH_3$), 3.40—3.84 (2H, m, 3α- and pyranose-C_5–H), 4.22 (2H, m, pyranose-C_6–H), 4.66 (1H, d, J=7 Hz, pyranose-C_1–H), 4.92—5.28 (3H, m, pyranose-C_2–, C_3– and C_4–H), 5.40 (1H, m, 5-H).

5α-Cholestan-3β-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (Vb)——Yield 42%. mp 175—176°. $[\alpha]_D^{21}$ −10.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.75; H, 9.48. NMR ($CDCl_3$) δ: 0.66 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.88 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.92 (3H, d, J=7 Hz, 21-CH_3), 2.02, 2.04, 2.06, 2.10 (12H, s, -$OCOCH_3$), 3.40—3.80 (2H, m, 3α- and pyranose-C_5–H), 4.16 (2H, m, pyranose-C_6–H), 4.62 (1H, d, J=7 Hz, pyranose-C_1–H), 4.84—5.30 (3H, m, pyranose-C_2–, C_3– and C_4–H).

5α-Cholestan-3α-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (Vc)——Yield 37%. mp 175—176°. $[\alpha]_D^{21}$ +10.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{66}O_{10}$: C, 68.49; H, 9.25. Found: C, 68.21; H, 9.24. NMR ($CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 0.76 (3H, s, 19-CH_3), 0.83 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.90 (3H, d, J=7 Hz, 21-CH_3), 2.04, 2.06, 2.10 (12H, s, -$OCOCH_3$), 3.70 (1H, m, pyranose-C_5–H), 3.95 (1H, m, 3β-H), 4.20 (2H, m, pyranose-C_6–H), 4.58 (1H, d, J=7 Hz, pyranose-C_1–H), 4.94—5.32 (3H, m, pyranose-C_2–, C_3– and C_4–H).

Cholest-5-en-3β-yl-β-D-glucopyranoside (VIa)——Yield 64%. mp 260—263° (dec.). $[\alpha]_D^{20}$ −20.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{33}H_{56}O_6$: C, 72.22; H, 10.29. Found: C, 71.92; H, 10.45. NMR (DMSO-d_6) δ: 0.67 (3H, s, 18-CH_3), 0.84 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.96 (3H, s, 19-CH_3), 4.23 (1H, d, J=7 Hz, pyranose-C_1–H), 5.30 (1H, m, 5-H). Reported mp 274°.[17]

5α-Cholestan-3β-yl-β-D-glucopyranoside (VIb)——Yield 68%. mp 240—244° (dec.). $[\alpha]_D^{21}$ −40.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{33}H_{58}O_6$: C, 71.96; H, 10.61. Found: C, 71.83; H, 10.72. NMR (DMSO-d_6) δ: 0.60 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.90 (6H, d, J=7 Hz, 26- and 27-CH_3), 4.16 (1H, d, J=7 Hz, pyranose-C_1–H).

5α-Cholestan-3α-yl-β-D-glucopyranoside (VIc)——Yield 42%. mp 220—221° (dec.). $[\alpha]_D^{21}$ −10.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{33}H_{58}O_6$: C, 71.96; H, 10.61. Found: C, 71.90; H, 10.89. NMR (DMSO-d_6) δ: 0.62 (3H, s, 18-CH_3), 0.75 (3H, s, 19-CH_3), 0.85 (6H, d, J=7 Hz, 26- and 27-CH_3), 4.12 (1H, d, J=7 Hz, pyranose-C_1–H).

Cholest-5-en-3β-yl-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (VIIa)——Yield 38%. mp 199—202°. $[\alpha]_D^{21}$ −20.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{65}NO_9$: C, 68.78; H, 9.15; N, 1.96. Found: C, 68.53; H, 9.08; N, 1.91. NMR ($CDCl_3$) δ: 0.69 (3H, s, 18-CH_3), 0.89 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.92 (3H, d, J=7 Hz, 21-CH_3), 1.00 (3H, s, 19-CH_3), 2.00, 2.08, 2.12 (12H, s, -$COCH_3$), 3.36—3.92 (3H, m, 3α-, pyranose-C_2– and C_5–H), 4.22 (2H, m, pyranose-C_6–H), 4.88 (1H, d, J=7 Hz, pyranose-C_1–H), 4.98—5.68 (4H, m, 5-, pyranose-C_3–, C_4– and N–H).

5α-Cholestan-3β-yl-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (VIIb)——Yield 36%. mp 248°. $[\alpha]_D^{21}$ −20.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{67}NO_9$: C, 68.58; H, 9.41; N, 1.95. Found: C, 68.40; H, 9.41; N, 1.93. NMR ($CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 0.78 (3H, s, 19-CH_3), 0.87 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.90 (3H, d, J=6 Hz, 21-CH_3), 1.93, 2.02, 2.07 (12H, s, -$COCH_3$), 3.40—3.80 (3H, m, 3α-, pyranose-C_2– and C_5–H), 4.14 (2H, m, pyranose-C_6–H), 4.82 (1H, d, J=7 Hz, pyranose-C_1–H), 4.92—5.53 (3H, m, pyranose-C_3–, C_4– and N–H).

5α-Cholestan-3α-yl-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (VIIc)——Yield 35%. mp 184—185°. $[\alpha]_D^{21}$ +10.0° (c=0.1, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{67}NO_9$: C, 68.58; H, 9.41; N, 1.95. Found: C, 68.33; H, 9.23; N, 1.83. NMR ($CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 0.76 (3H, s, 19-CH_3), 0.88 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.90 (3H, d, J=6 Hz, 21-CH_3), 1.96, 2.04, 2.06 (12H, s, -$COCH_3$), 3.60—3.88 (2H, m, pyranose-C_2– and C_5–H), 3.94 (1H, m, 3β-H), 4.19 (2H, m, pyranose-C_6–H), 4.78 (1H, d, J=7 Hz, pyranose-C_1–H), 4.96—5.58 (3H, m, pyranose-C_3–, C_4– and N–H).

Cholest-5-en-3β-yl-2-acetamido-2-deoxy-β-D-glucopyranoside (VIIIa)——Yield 93%. mp 192—194°. $[\alpha]_D^{21}$ +30.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{35}H_{59}NO_6 \cdot H_2O$: C, 69.15; H, 10.12; N, 2.30. Found: C, 69.45; H, 10.28; N, 2.34. NMR (DMSO-d_6) δ: 0.66 (3H, s, 18-CH_3), 0.86 (6H, d, J=7 Hz, 26- and 27-CH_3), 0.96 (3H, s, 19-CH_3), 1.82 (3H, s, -$COCH_3$), 5.34 (1H, m, 5-H), 7.60 (1H, m, N–H).

18) J. Mckenna and J.K. Norymberski, *J. Chem. Soc.*, **1957**, 3893.

5α-Cholestan-3β-yl-2-acetamido-2-deoxy-β-D-glucopyranoside (VIIIb)——Yield 53%. mp 192—193°. $[\alpha]_D^{21}$ −10.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{35}H_{61}NO_6 \cdot 3/4H_2O$: C, 69.44; H, 10.41; N, 2.31. Found: C, 69.37; H, 10.39; N, 2.35. NMR (DMSO-d_6) δ: 0.64 (3H, s, 18-CH_3), 0.73 (3H, s, 19-CH_3), 0.84 (6H, d, J=7 Hz, 26- and 27-CH_3), 1.80 (3H, s, -$COCH_3$), 7.60 (1H, m, N-H).

5α-Cholestan-3α-yl-2-acetamido-2-deoxy-β-D-glucopyranoside (VIIIc)——Yield 73%. mp 190—191°. $[\alpha]_D^{21}$ +30.0° (c=0.1, MeOH). *Anal.* Calcd. for $C_{35}H_{61}NO_6 \cdot 1/2H_2O$: C, 69.96; H, 10.40; N, 2.33. Found: C, 69.75; H, 10.21; N, 2.44. NMR (DMSO-d_6) δ: 0.62 (3H, s, 18-CH_3), 0.74 (3H, s, 19-CH_3), 0.86 (6H, d, J=7 Hz, 26- and 27-CH_3), 1.76 (3H, s, -$COCH_3$), 7.58 (1H, m, N-H).

(6,7α,7β-d_3-Cholest-5-en-3β-yl-β-D-glucopyranosid)uronic Acid (IX)——6,7α,7β-d_3-Cholesterol (103 mg, 90% d_3), obtainable by the method of Wyllie *et al.*,[19] was treated in the manner as described for IIIa. mp 228—230° (dec.). Mixed melting point on admixture with a non-labeled authentic sample showed no depression. Mass spectrum (m/e 372[M-423]$^+$) showed the following isotopic composition: 0% d_0, 2% d_1, 11% d_2 and 87% d_3.

Gas Chromatography-Mass Spectrometry

Non-deuterated and deuterated cholesterol glucuronides (IIIa, IX) were analyzed as the methyl ester-trimethylsilyl (TMS) ether derivatives. IIIa or IX (100 μg) was dissolved in MeOH and methylated with diazomethane in the usual manner. The methyl ester was then treated with TMS imidazole (50 μl) in pyridine (50 μl) at 50° for 1 hr. The apparatus used for electron impact mass spectrometry was a JEOL JMS-01SG-2 g.c.m.s. system equipped with a JEOL JMA-2000 computer. A coiled glass column (1 m×2 mm i.d.) was packed with 3% OV-1 on Gas Chrom Q (100—120 mesh) and used at 300°. The temperatures of the separator and ion source were 280° and 270°, respectively. The electron energy was 75 eV.

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A Convenient Synthesis of α-Substituted Cyclic α-Imino Acids[1]

HARUNORI YASUO, MAMORU SUZUKI, and NAOTO YONEDA

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd.[2]

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Various α-substituted α-imino acids (**6**) were synthesized in good yields by the reaction of methyl α-substituted α-isocyanoacetates with alkylene bromides in the presence of sodium hydride, followed by cyclization and hydrolysis.

Keywords——methyl α-isocyanoacetates; alkylene bromides; α-substituted cyclic α-imino acid methyl esters; α-substituted cyclic α-imino acids; alkylation; ring transformation

Various cyclic α-imino acids such as proline and pipecolic acid are not only important constituents of proteins but are also pharmacologically interesting as intermediates in the preparation of drugs. On the other hand, the corresponding α-substituted cyclic α-imino acids have hardly been investigated and only a few methods for their syntheses have been

1) This study was presented at the 28th Annual Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Nishinomiya, October 1978.

2) Location: *16-89 Kashima-3-chome, Yodogawa-ku, Osaka 532, Japan.*