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Synthesis of (24*R*)- and (24*S*)-27-Nor-5β-cholestane-3α,7α,12α,24,26-pentols[1]

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(24*R*)- and (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentols were synthesized, starting from 3α,7α,12α-trihydroxy-5β-cholan-24-al, by means of the Reformatsky reaction with bromoacetate and subsequent lithium aluminum hydride reduction of the resulting (24*R*)- and (24*S*)-3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oates. The configurations at C-24 of the synthetic pentols were assigned by ^{13}C-nuclear magnetic resonance spectroscopy. By direct comparison with a synthetic specimen, 5β-ranol, the major bile constituent of bullfrog, was shown to be (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentol. The availability of the 24*S*-epimer of 5β-ranol enabled us to ascertain the absence of this bile alcohol in the bullfrog bile.

Keywords——synthesis; bile alcohol; bullfrog; structural determination; (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentol; (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentol; ^{13}C-NMR; 5β-ranol

A bile alcohol, 5β-ranol, is the major bile constituent of the bullfrog, *Rana catesbeiana*.[2] The chemical structure of 5β-ranol has been determined to be 27-nor-5β-cholestane-3α,7α,12α,24,26-pentol.[3] However, the stereochemistry at C-24 remains to be established. Two minor bile alcohols have been isolated from the bullfrog bile and characterized as (24*R*)- and (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrols.[4] The structural relationship between these tetrols and 5β-ranol suggests that one of the former compounds is the direct biosynthetic precursor of the latter.[5] The occurrence of both 24-epimeric tetrols raises the question of whether or not the 24-epimer of 5β-ranol occurs in the bullfrog bile. This question, as well as the assignment of the configuration at C-24 of 5β-ranol, could be better answered if authentic 24-*epi*-5β-ranol were available. We now report the synthesis of (24*R*)- and (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentols.

The synthetic route to the pentols epimeric at C-24 is shown in Chart 1. 3α,7α,12α-Trihydroxy-5β-cholan-24-al (I) prepared from cholic acid according to the method of Yashima[6] was converted into the tetrahydropyranyl derivative because of the poor solubility of the aldehyde (I) in benzene and toluene. Treatment of the tetrahydropyranyl derivative of I with an excess of ethyl bromoacetate and zinc in benzene and toluene followed by acid hydrolysis and alkaline hydrolysis provided a mixture of 24-epimeric 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acids (IIa and IIb). The mixture was treated with ethereal diazomethane and the resulting methylated mixture was chromatographed on a silica gel column to obtain IIIa, mp 183.5°C, $[\alpha]_D$ +35°, and IIIb, mp 162.5—163.5°C, $[\alpha]_D$ +30°. The less polar ester, IIIa, was treated with lithium aluminum hydride in tetrahydrofuran to give IVa, mp 178—179°C, $[\alpha]_D$ +35°. By the same procedure, the more polar ester, IIIb was converted to IVb, mp 195.5—196.5°C, $[\alpha]_D$ +30°. The structures of IVa and IVb as the 24-epimeric pair of 27-nor-5β-cholestane-3α,7α,12α,24,26-pentol were confirmed by spectral analysis. Mass spectra (MS) of the trimethylsilyl ether derivatives of IVa and IVb were essentially identical to each other. The spectra were characterized by a molecular ion at *m/z* 798 and a series of peaks at *m/z* 708, 618, 528, 438, and 348, resulting from the successive loss of one, two, three, four, and five molecules of trimethylsilanol from the molecular ion. There was another series of peaks at *m/z* 681, 591, 501, 411, and 321, resulting from the scission of the bond between C-24 and C-25, and the consecutive loss of one, two, three, and four molecules of trimethylsilanol.

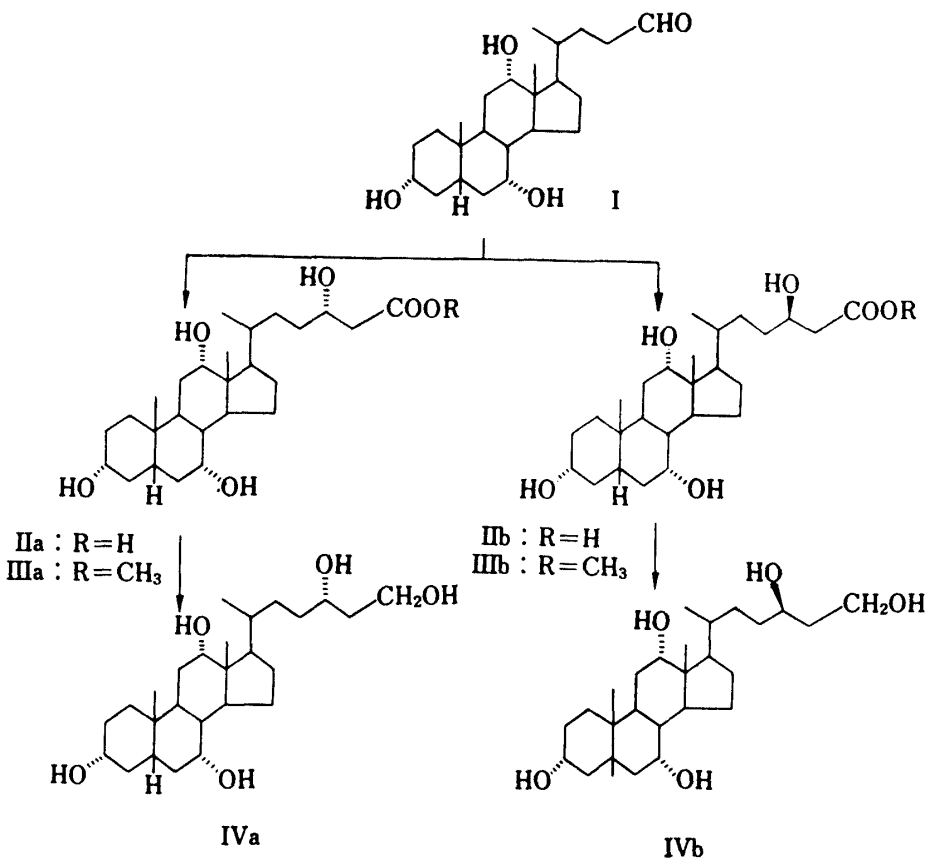

Chart 1. Synthesis of 27-Norcholestanepentols

Peaks at *m/z* 219, and 129 are side chain fragments, resulting from the scission of the bond between C-23 and C-24, and the loss of a trimethylsilanol molecule. The base peak at *m/z* 103 is a side chain fragment, resulting from scission of the bond between C-25 and C-26. The ions at *m/z* 433, 343, and 253 represent loss of the side chain plus one, two, and three nuclear trimethylsilyl ether groups from the molecular ion. The proton nuclear magnetic resonance (PMR) spectrum of IVa showed the presence of three methyl groups and six carbinyl protons. The PMR spectrum of IVb was identical with that of IVa, except for the chemical shift differences in the resonances of the C-19 and C-26 methyls. The infrared (IR) spectra of IVa and IVb did not differ significantly and each exhibited a strong hydroxyl band at 3400 cm^{-1} and a series of bands in the fingerprint region characteristic of the cholic acid-type nucleus.[7]

The configurations at C-24 of IVa and IVb were assigned by carbon-13 nuclear magnetic resonance (CMR) spectroscopy (Table I). A small difference in the CMR spectra of these

TABLE I. ^{13}C-Chemical Shifts of the Side-chain Carbons of Synthetic and Natural 5β-Ranols, and Ranol Fraction

Carbon No.	IVb (24*R*)	IVa (24*S*)	Natural 5β-ranol	Ranol fraction
20	36.2	36.2	36.3	36.3
21	18.0	18.1	18.0	18.0
22	32.4	32.5	32.4	32.4
23	35.2	35.3	35.1	35.2
24	70.1	70.5	70.1	70.1
25	40.7	40.7	40.7	40.7
26	60.4	60.4	60.4	60.4

epimers was observed in the chemical shift of C-24, which was 70.5 ppm for IVa and 70.1 ppm for IVb. It has been reported that among several pairs of 24-epimeric steroids the C-24 resonance in the 24*R* compounds is always at slightly higher field than in the 24*S* isomers.[8] Though the difference is small, it can be used for stereochemical assignment when the epimers can be compared.[8] Thus, it was concluded that the less polar epimer, IVa has the *S* configuration at the C-24 position, and the more polar one, IVb, has the *R* configuration.

By direct comparison with the specimen synthesized in the present work, 5β-ranol isolated from the bullfrog bile was shown to be (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentol (IVb). The natural bile alcohol had the same melting point, optical rotation, chromatographic properties, IR, PMR, CMR (Table I), and MS as the synthetic 24*R*-compound (IVb).

In order to ascertain whether or not the 24*S*-epimer of 5β-ranol occurs in the bullfrog bile, the bile alcohols of this frog was re-examined. Acid hydrolysis of the ethanolic extract of the bullfrog bile afforded a mixture of bile alcohols. The mixture was fractionated by reversed-phase partition chromatography to give a fraction from which 5β-ranol was isolated by crystallization. Since 24-epimeric bile alcohols have very similar chromatographic properties,[9] 24-*epi*-5β-ranol should be eluted together with 5β-ranol from the column and contained in the mother liquor separated from the crystals of 5β-ranol. As described above, 5β-ranol and its 24*S*-epimer can be distinguished by the difference of ^{13}C-chemical shifts at C-24. Thus, the mother liquor was concentrated and the residue (ranol fraction) was analyzed by CMR spectroscopy. In the CMR spectrum of the residue, the chemical shifts of signals of the side chain carbons, C-20—C-26, were identical with those of 5β-ranol (Table I). The C-24 signal appeared at 70.1 ppm and no signal at 70.5 ppm was observed. The results strongly suggest that essentially no 24-*epi*-5β-ranol is present in the bullfrog bile. It seems, therefore, reasonable to suppose that only (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (26-deoxy-5β-ranol), not its 24*R*-epimer (24-epi-26-deoxy-5β-ranol), can be hydroxylated at C-26 in the bullfrog.

Experimental

Melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. IR spectra were taken on a JASCO IRA-1 spectrometer as KBr discs. PMR and CMR spectra were obtained at 100 MHz on a JEOL JNM PS-100 spectrometer using pyridine-d_5 as the solvent, and chemical shifts are given in the δ ppm scale with tetramethylsilane as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), and m (multiplet). Molecular weights were determined from the molecular ion in high-resolution mass spectra, which were recorded on a JEOL JMS-01SG-2 mass spectrometer with an accelerating potential of 9.45 kV, an ionization potential of 75 eV and a source temperature of 120—140°C. Gas–liquid chromatography (GLC) was run on a Shimadzu GC-6A gas chromatograph using glass columns (2 m × 4 mm) packed with 2% OV-1, 2% OV-17, or 3% QF-1 on Gas-Chrom Q (80—100 mesh). Trimethylsilyl (TMS) ethers were prepared with hexamethyldisilazane and trimethylchlorosilane in pyridine at room temperature. Retention times are reported relative to the TMS derivative of methyl cholate. Thin-layer chromatography (TLC) was carried out on Silica gel G plates using a 10% solution of phosphomolybdic acid in EtOH as the detection reagent. The following solvent systems were used: CAM-1 (chloroform–acetone–methanol, 140: 60: 3 by vol.), and CAM-2 (chloroform–acetone–methanol, 70: 30: 3 by vol.). Reversed-phase partition column chromatography was performed according to the procedure reported previously.[10]

Methyl (24*S*)- and (24*R*)-3α,7α,12α,24-Tetrahydroxy-27-nor-5β-cholestan-26-oates (IIIa and IIIb)——3α,7α,12α-Trihydroxy-5β-cholan-24-al (I) (1 g) was dissolved in 10 ml of dihydropyran containing two drops of conc.-HCl with vigorous shaking. The reaction mixture was allowed to stand for 2 h and then diluted with ether. The solution was washed with water until free from hydrochloric acid and the solvent was evaporated to dryness. A solution of the resulting residue in 10 ml of benzene was added to a mixture of 10 ml of benzene, 20 ml of toluene, and 1 ml of ethyl bromoacetate, containing 500 mg of granulated zinc, a few crystals of iodine, and a small amount of powdered copper. The reaction mixture was refluxed for 2 h. After cooling to 0°C in ice, the reaction mixture was poured into a mixture of crushed ice and 10% sulfuric acid to decompose the Reformatsky product. The solution was extracted with three 100 ml portions of ethyl acetate. The extracts were combined and washed with water, 2% $Na_2S_2O_3$, water, 2% $NaHCO_3$, and water successively, dried over Na_2SO_4 and then evaporated to dryness. The residue was refluxed with 10 ml of 70% acetic acid for an hour and the solvent was evaporated off under reduced pressure. The resutling

residue was refluxed with 50 ml of 10% methanolic KOH for 2 h. The methanol was evaporated off, water was added to the residue, and the alkaline solution was extracted with ether in order to remove the unreacted aldehyde. The aqueous layer was acidified to pH 1 with dilute HCl and extracted with three 100 ml portions of ethyl acetate. The extracts were combined, washed with water, dried over Na_2SO_4 and then evaporated to dryness. A solution of the residue in methanol was treated with ethereal diazomethane solution. After 2 h, the solvents and excess diazomethane were driven off by gentle heating. The resulting methylated material was chromatographed over a Silica gel 60 pre-packed column (2.5 cm × 25 cm, Merck) using $CHCl_3$–acetone–MeOH (14: 4: 1 by vol.) as the eluting solvent. The column effluents were monitored by TLC. Fractions No. 31—No. 42 (15 ml/tube) gave a product which was crystalized from MeOH–AcOEt to yield crystals (252 mg) of methyl (24*S*)-3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oate (IIIa). mp 183.5—184.5°C. $[\alpha]_D$ +35° (c=1.3, MeOH). M^+, 466.3346 (Calcd for $C_{27}H_{46}O_6$, 466.3294). TLC 0.57 (CAM-1). GLC (relative *ts*) 2.45 (2% OV-1), 1.86 (3% QF-1). IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl), 1710 (ester). PMR: 0.76 (s, 3H, 18-CH_3), 0.95 (s, 3H, 19-CH_3), 1.17 (d, J=6 Hz, 3H, 21-CH_3), 3.45 (s, 3H, -$COOCH_3$), 3.58 (m, 1H, C-3βH), 3.94 (m, 1H, C-7βH), 4.08 (m, 1H, C-12βH), 4.20 (m, 1H, C-24H). Fractions (No. 52—No. 66) gave a product which was crystallized from ethyl acetate to yield crystals (212 mg) of methyl (24*R*)-3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oate (IIIb). mp 162.5—163.5°C. $[\alpha]_D$ +30° (c=1.3 MeOH). M^+ 466.3389 (Calcd for $C_{27}H_{46}O_6$, 466.3294). TLC 0.46 (CAM-1). GLC 2.47 (2% OV-1), 1.88 (3% QF-1). IR ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl), 1710 (ester). PMR: 0.77 (s, 3H, 18-CH_3), 0.97 (s, 3H, 19-CH_3), 1.18 (d, J=6 Hz, 3H, 21-CH_3), 3.45 (s, 3H, -$COOCH_3$), 3.62 (m, 1H, 3βH), 3.94 (m, 1H, 7βH), 4.07 (m, 1H, 12H), 4.20 (m, 1H, 24H).

(24*S*)- and (24*R*)-27-Nor-5β-cholestane-3α,7α,12α,24,26-pentols (IVa and IVb)——$LiAlH_4$ (0.1 g) was added to a solution of 120 mg of the methyl (24*S*)-3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oate (IIIa) in 10 ml of tetrahydrofuran at 0°C, and the reaction mixture was refluxed for 5 h. The whole was cooled to 0°C, then crushed ice (500 g) and 2 N H_2SO_4 (20 ml) were added to decompose the excess $LiAlH_4$. The precipitated product was extracted with four 100 ml portions of ethyl acetate. Evaporation of the solvent from the washed and dried extracts left a residue, which was recrystallized from methanol–ethyl acetate to give crystals (75 mg) of (24*S*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentol (IVa). mp 178—179°C. $[\alpha]_D$ +35° (c=1.00 MeOH). M^+ 438.3342 (Calcd for $C_{26}H_{46}O_5$, 438.3344). TLC 0.53 (CAM-2). GLC 2.18 (2% OV-1), 1.55 (2% OV-17), 1.23 (3% QF-1). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl). PMR: 0.82 (s, 3H, 18-CH_3), 1.01 (s, 3H, 19-CH_3), 1.25 (d, J=6 Hz, 3H, 21-CH_3), 3.74 (m, 1H, C-3βH), 4.22 (t, 2H, 26-CH_2OH), 4.10—4.28 (m, 3H, C-7βH, C-12H, C-24H). The (24*R*)-ester, IIIb (200 mg) was converted to (24*R*)-27-nor-5β-cholestane-3α,7α,12α,24,26-pentol (IVb) (100 mg) in the same way as described above, mp 195.5—196.5°C (methanol-ethyl acetate). $[\alpha]_D$ +30° (c=1.02 MeOH). M^+, 438.3417 (Calcd for $C_{26}H_{46}O_5$, 438.3344). TLC 0.52 (CAM-2). GLC 2.18 (2% OV-1), 1.55 (2% OV-17), 1.23 (3% QF-1). IR ν_{max}^{KBr} cm^{-1}: 3400 (hydroxyl). PMR: 0.82 (s, 3H, 18-CH_3), 0.98 (s, 3H, 19-CH_3), 1.25 (d, J=6 Hz, 3H, 21-CH_3), 3.72 (m, 1H, C-3βH), 4.18 (t, 2H, 26-CH_2OH), 4.10—4.30 (m, 3H, C-7βH, C-12βH, C-24H).

Isolation of 5β-Ranol and Detection of 24-*epi*-5β-ranol (IVa) in the Bullfrog Bile——Gallbladder bile of bullfrog, *Ranacatesbeiana*, was extracted with 20 volumes of ethanol, to yield crude bile salts. After evaporation of the ethanol, the bile salts (800 mg) were dissolved in water, acidified with dilute HCl and extracted with ether in order to remove unconjugated bile acids. The aqueous layer was evaporated to dryness, and the residue containing bile alcohol sulfate was subjected to acid hydrolysis by a modification of the method described previously.[11] The residue (650 mg) was heated with 60 ml of a mixture (1: 1) of acetic acid and acetic anhydride on a water bath for 1 h, and the solvents were evaporated off under reduced pressure. The residue was dissolved in 60 ml of a 40% solution of trichloroacetic acid in dioxane. After standing at room temperature for 2 weeks, the reaction mixture was diluted with water (200 ml) and extracted with ethyl acetate (200 ml × 3). The ethyl acetate extracts were combined, washed with 2% Na_2CO_3 solution (100 ml × 2) and water, dried over Na_2SO_4, and evaporated to dryness. The residue was hydrolyzed with 1 N methanolic KOH on a boiling water bath for 1 h. The hydrolysate was diluted with water and extracted with a mixture (1: 1) of ethyl acetate and *n*-butanol. The extract was washed with water, dried over Na_2SO_4, and evaporated to dryness, and the residue was subjected to reversed-phase column partition chromatography, using a mixture (1: 1) of chloroform and 2-ethylhexyl alcohol as the stationary phase and 50% aqueous methanol as the moving phase. A glass column was packed with 40.5 g of Hostalene (polyethylene powder, Farbwerke Hoechst, Germany) supporting 27 ml of the stationary phase. The sample was mixed with 3 ml of the stationary phase and 4.5 g of Hostalene and applied to the column. The column effluents were monitored by TLC. 5β-Ranol was eluted in effluents from 180 ml to 315 ml, and these were combined and evaporated to dryness. The residue was crystallized from methanol–ethyl acetate to give crystals (155 mg) of 5β-ranol, mp 195—196°C (methanol–ethyl acetate). $[\alpha]_D$ +30° (c=1.00, MeOH). M^+, 438.3398 (Calcd for $C_{26}H_{46}O_5$, 438.3344). TLC 0.53 (CAM-2), GLC 2.18 (2% OV-1), 1.54 (2% OV-17), 1.23 (3% QF-1). IR ν_{max}^{KBr} cm^{-1} : 3400 (hydroxyl). PMR 0.82 (s, 3H, 18-CH_3), 0.98 (s, 3H, 19-CH_3), 1.25 (d, J=6 Hz, 3H, 21-CH_3), 3.72 (m, 1H, C-3βH), 4.18 (t, 2H, 26-CH_2OH), 4.10—4.30 (m, 3H, C-7βH, C-12βH, C-24αH). The mother liquor was concentrated to dryness and the resulting residue (ranol fraction) (85 mg) was analyzed by CMR spectroscopy.

References and Notes

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