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Synthesis of (22*R*)- and (22*S*)-22-Hydroxylanosterols[1)]

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Epoxidation of (22*E*)-3β-acetoxylanosta-8,22-dien-24-one (**1**) afforded the 22,23-epoxides (**2**) which were led to (22*R*)-22-hydroxylanosterol (**7a**) predominantly and (22*S*)-22-hydroxylanosterol (**8a**) as a minor product in a few steps. The epimeric relationship of **7a** and **8a** was confirmed by Jones oxidation of **7a** to give the 3,22-diketo compound (**9**), which furnished mainly (22*S*)-22-hydroxylanosterol (**8a**) on hydride reduction.

Keywords——(22*R*)-22-hydroxylanosterol; (22*S*)-22-hydroxylanosterol; lanosta-8,24-diene-3,22-dione; hydrazine hydrate; pyrazole compound; inotodiol

We have recently shown[1)] that some lanosterol analogs with modified side chains inhibit cholesterol biosynthesis from lanosterol. In this connection, 22-hydroxylated lanosterol analogs were required for the investigation of the biological activity. (22*R*)-22-Hydroxylanosterol (inotodiol),[2)] which was isolated from the birch fungus *Inonotus obliquus*, has been synthesized by Poyser *et al.*[2)] This paper describes the synthesis of (22*R*)- and (22*S*)-22-hydroxylanosterols.

The starting material, (22*E*)-3β-acetoxylanosta-8,22-dien-24-one (**1**, Chart 1) in this study, was prepared from lanosterol according to the published procedure.[3)] Reaction of **1** with H_2O_2 in an alkaline solution gave the epoxy compounds (**2**) which exhibited one spot on thin layer chromatography (TLC). As epoxidation of this type is reported[4,5)] to give the (22*S*, 23*R*)-epoxy compound, the main component of **2** is expected to have the (22*S*, 23*R*)-configuration although four stereoisomers are possible in keto epoxides. Treatment of **2** with hydrazine hydrate[4,6)] in EtOH gave an undesired nitrogen-containing compound. The structure of the latter compound was assumed to be **3a** or **3b** from its mass spectrum (MS) (*m/z* 494, M^+), ele-

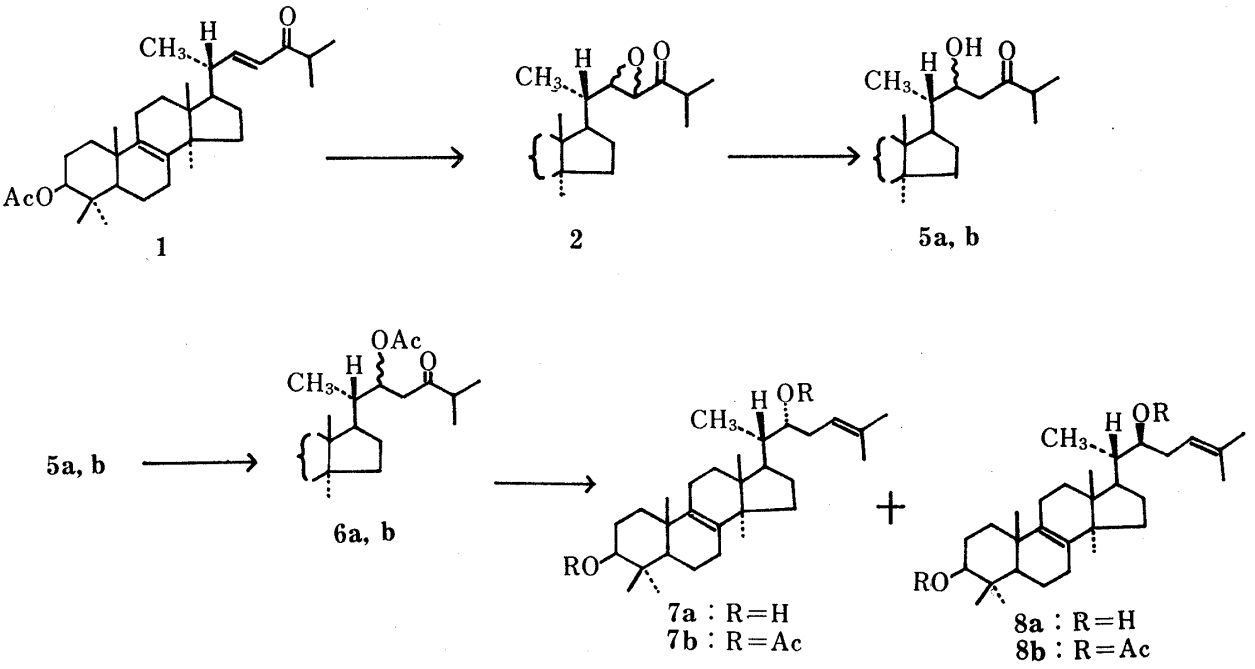
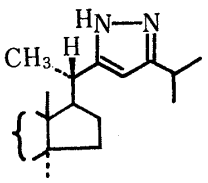


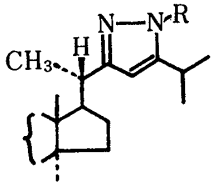
Chart 1

mental analysis, and the proton nuclear magnetic resonance (PMR) spectrum, which showed a six-proton doublet at 1.24 ppm due to the 26,27-methyl groups and a singlet at 5.76 ppm due to 23-H. However, treatment of **3** with Ac_2O–pyridine gave an acetylated product which in the PMR spectrum showed a downfield shift of the heptet signal assignable to 25-H from 2.86 to 3.67 ppm, suggesting its structure as **4**. This type of pyrazole[7] has been reported in the reaction of 16α,17α-epoxy-3β-hydroxypregn-5-en-20-one with hydrazine hydrate.

Therefore, **2** was reacted with hydrazine hydrate and potassium carbonate in dimethylsulfoxide (DMSO)[8] at room temperature to furnish the 22ξ-hydroxy-24-keto compounds (**5a, b**). In the PMR spectrum of the latter compounds, the signal of a proton attached to C-22 (carrying the hydroxy group) was observed at 4.03—4.25 ppm as a multiplet and those of three protons adjacent to the 24-keto group were observed at 2.40—2.72 ppm as multiplets. The reaction probably proceeds as follows. That is, the 24-hydrazone of **2** is formed and the epoxy bond is cleaved at C-23 to give an intermediate as shown in Fig. 2, and finally this is transformed to the 22-hydroxy-24-keto compound with evolution of diimide by the action of alkali.



3a



3b : R=H
4 : R=Ac

Fig. 1

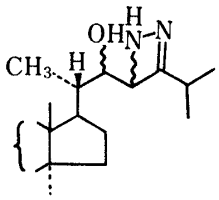
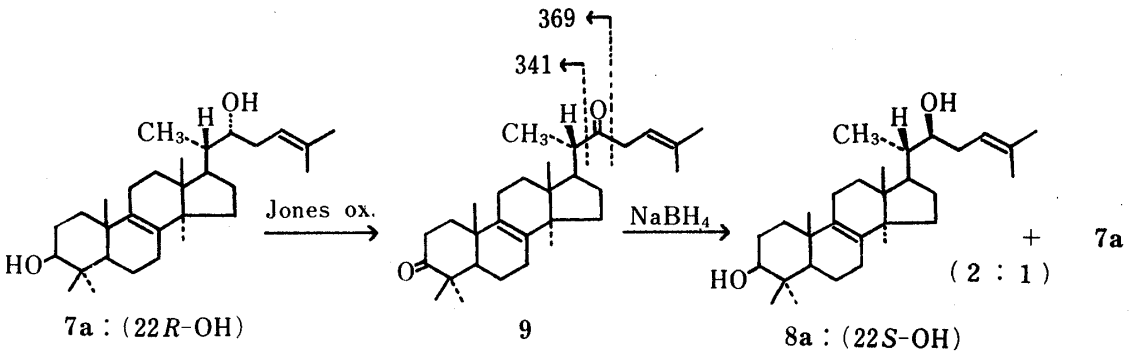


Fig. 2

Next, **5a, b** was acetylated as usual to give the corresponding acetates (**6a, b**), which were successively treated with sodium borohydride and with phosphoryl chloride. By TLC examination, the crude products were proved to be composed of two components, which were separated by column chromatography on silica gel. The less polar major product was considered to be (22*R*)-3β,22-diacetoxylanosta-8,24-diene (**7b**) by comparison of its properties with the published data[2] (mp, PMR, MS, and $[\alpha]_D$). Compound **7b** was hydrolyzed with alkali to give (22*R*)-22-hydroxylanosterol (**7a**), whose physical and spectral data were also consistent with the published data.[2] Therefore, the more polar minor product was considered to be the C-22 epimer of **7b**, (22*S*)-3β,22-diacetoxylanosta-8,24-diene (**8b**), on the basis of PMR and MS analyses. Alkaline hydrolysis of **8b** furnished (22*S*)-22-hydroxylanosterol (**8a**).

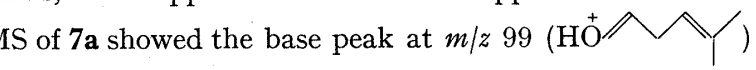
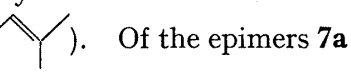
That these compounds (**7a** and **8a**) were the C-22 epimers was confirmed by Jones oxidation of **7a** to give the diketo compound (**9**), followed by sodium borohydride reduction (Chart 2). The resulting mixture of alcohols contained **8a** and **7a** in a ratio of 2: 1[2] as analyzed by gas liquid chromatography (GLC). The MS of **9** showed *m/z* 369 (M^+ −69) and 341 (M^+ −97), which may result from fissions of the 22—23 and 20—22 bonds, respectively, characteristic



7a : (22*R*-OH) **9** **8a** : (22*S*-OH)

Chart 2

of the 22-ketone.

Some chemical-shift differences[3] in the PMR spectra of the C-22 epimers (**7a** and **8a**) were observed at the 32- and 18-methyl signals, which appeared at 0.88 and 0.72 ppm for **7a** and 0.91 and 0.70 ppm for **8a**, respectively. MS of **7a** showed the base peak at *m/z* 99 () as observed previously.[2] Further, a peak at *m/z* 99 has been described in the MS of (22*R*)-22-hydroxydesmosterol.[9] On the other hand, MS of **8a** showed the base peak at *m/z* 69 (). Of the epimers **7a** and **8a**, the (22*R*)-hydroxy compound (**7a**) showed, as expected, more positive molecular rotation than the (22*S*)-epimer, as observed in the 22-hydroxycholesterol series.[9] In GLC on 1.5% OV-17, the relative retention times of **7a** and **8a** with respect to lanosterol were 2.1 and 2.0, respectively.

The biological activities of the 22-epimeric 22-hydroxylanosterols (**7a** and **8a**) will be reported elsewhere.

Experimental

All melting points were obtained on a micro-melting point determination apparatus and are uncorrected. PMR spectra were recorded on a JEOL JNM-MH-100 spectrometer at 100 MHz, with tetramethylsilane as an internal standard in deuteriochloroform. Abbreviations used: s=singlet, d=doublet, br d=broad doublet, h=heptet, m=multiplet. IR spectrum was recorded on a Hitachi EPI-G3 machine as KBr pellet. Mass spectra (MS) were determined on a JEOL JMS D-100 spectrometer at an ionizing voltage of 75 eV. Column chromatography was performed on silica gel (Kanto Kagaku, 100 mesh above) and aluminum oxide 90 (E. Merck, Darmstadt). Thin layer chromatography was done on Merck precoated Kieselgel 60 F_{254} plates (0.25 mm thick). Gas liquid chromatography was performed on a Shimadzu GC-6AF machine with a 1.5% OV-17 (2 m×3 mm) column. Optical rotations were determined on a JASCO DIP-SL machine and are recorded as follows, $[\alpha]_D^{temp.}$ (% concentration, solvent). "The usual work-up" refers to dilution of the reaction mixture with water, extraction with CH_2Cl_2, washing of the extract with water, drying over Na_2SO_4, and concentration under reduced pressure.

3β-Acetoxy-22ξ,23ξ-epoxylanost-8-en-24-one (**2**)——Hydrogen peroxide (30%, 12 ml) and 4 N NaOH (2 ml) were added to a solution of **1** (0.5 g) in MeOH (200 ml) and the whole was stirred for 2 h at 30°C. The reaction mixture was poured into H_2O and extracted with methylene chloride. The organic layer was washed with 10% $NaHSO_3$ and H_2O, dried and concentrated. The residue was chromatographed on aluminum oxide 90 (50 g). Elution with methylene chloride gave a solid (0.3 g) which was recrystallized from MeOH to give colorless needles of **2** (255 mg), mp 173—174°C. *Anal.* Calcd for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 76.66; H, 9.85. MS *m/z*: 498 (M^+), 483, 423, 43 (base peak). PMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.10 (3H, d, 26- or 27-CH_3, J=6.5 Hz), 1.15 (3H, d, 26- or 27-CH_3, J=6.5 Hz), 2.04 (3H, s, 3-$OCOCH_3$), 2.58—2.96 (2H, m, 22- and 25-H), 3.28 (1H, d, 23-H, J=2 Hz), 4.36—4.64 (1H, m, 3-H).

Reaction of 2 with Hydrazine Hydrate——Hydrazine hydrate (90%, 0.2 g) in EtOH (10 ml) was added to a solution of **2** (0.5 g) in EtOH (15 ml) containing AcOH (20 mg), and the whole was stirred for 1.5 h at room temperature. After usual work-up, the residue was chromatographed on silica gel (50 g). Elution with methylene chloride gave a solid (0.35 g), which was recrystallized from MeOH to give colorless needles of the pyrazole compound (**3**), mp 248—250°C. *Anal.* Calcd for $C_{32}H_{50}N_2O_2$: C, 77.68; H, 10.19; N, 5.66. Found: C, 77.96; H, 10.28; N, 5.14. MS *m/z*: 494 (M^+), 479, 419, 137 (side chain, base peak). IR ν_{max}^{KBr} cm^{-1}: 1700 (C=N), 1738 (3-$OCOCH_3$), 3300 (N–H). PMR δ (ppm): 0.76 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.24 (6H, d, 26- and 27-CH_3, J=6.5 Hz), 2.02 (3H, s, 3-$OCOCH_3$), 2.64—3.08 (2H, m, 20- and 25-H), 4.36—4.60 (1H, m, 3-H), 5.76 (1H, s, 23-H), 7.36—7.60 (1H, N–H, exchangeable with D_2O).

Acetylation of 3——**3** was acetylated with Ac_2O–pyridine as usual to give the *N*-acetyl compound (**4**). Recrystallization from MeOH gave colorless needles of **4**, mp 152—154°C. *Anal.* Calcd for $C_{34}H_{52}N_2O_3$: C, 76.07; H, 9.77; N, 5.22. Found: C, 75.80; H, 9.73; N, 5.09. MS *m/z*: 536 (M^+), 521, 479, 461, 137 (base peak). PMR δ (ppm): 0.79 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.24 (3H, d, 26- or 27-CH_3, J=6.5 Hz), 1.26 (3H, d, 26- or 27-CH_3, J=6.5 Hz), 2.03 (3H, s, 3-$OCOCH_3$), 2.66 (3H, s, N–$COCH_3$), 2.70—3.00 (1H, m, 20-H), 3.67 (1H, h, 25-H), 4.40—4.64 (1H, m, 3-H), 6.03 (1H, d, 23-H, J=4 Hz).

3β-Acetoxy-22ξ-hydroxylanost-8-en-24-one (**5a, b**)——K_2CO_3 (0.2 g) and hydrazine hydrate (90%, 2.0 g) were added to a solution of **2** (recrystallized sample, 0.2 g) in DMSO (20 ml), and the whole was stirred for 2 h at room temperature. The reaction mixture was poured into H_2O and extracted with methylene chloride. The organic layer was washed with saturated NaCl solution, dried and concentrated. The residue was chromatographed on silica gel (50 g). Elution with methylene chloride gave a solid (0.15 g) which was

recrystallized from MeOH to give colorless needles of **5a**, **b**, mp 212—213°C. *Anal.* Calcd for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.90; H, 10.44. MS *m/z*: 500 (M^+), 485, 467, 407, 43 (base peak). PMR δ (ppm): 0.73 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.12 (6H, d, 26- and 27-CH_3 J=6.5 Hz), 2.03 (3H, s, 3-$OCOCH_3$), 2.40—2.72 (3H, m, 23-H_2 and 25-H), 4.03—4.25 (1H, m, 22-H), 4.40—4.64 (1H, m, 3-H).

3β,22ξ-Diacetoxylanost-8-en-24-one (6a, b)——**5a**, **b** was acetylated with Ac_2O–pyridine as usual to give **6a**, **b**. Recrystallization from MeOH gave colorless needles of **6a**, **b**, mp 213—214°C. *Anal.* Calcd for $C_{34}H_{54}O_5$: C, 75.23; H, 10.03. Found: C, 74.83; H, 9.95. MS *m/z*: 542 (M^+), 482, 467 (M^+-75, base peak), 407. PMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.08 (6H, d, 26- and 27-CH_3, J=6.5 Hz), 1.96 (3H, s, 22-$OCOCH_3$), 2.03 (3H, s, 3-$OCOCH_3$), 2.28—2.84 (3H, m, 23-H_2 and 25-H), 4.36—4.60 (1H, m, 3-H), 5.24—5.50 (1H, m, 22-H).

(22*R*)-3β,22-Diacetoxylanosta-8,24-diene (7b) and (22*S*)-3β,22-Diacetoxylanosta-8,24-diene (8b)——$NaBH_4$ (0.1 g) was added to a solution of **6a**, **b** (0.1 g) in MeOH–tetrahydrofuran (12 ml, 5: 1) and the mixture was stirred at room temperature for 2 h. The usual work-up afforded the crystalline alcohol. This crude alcohol was dissolved in pyridine (5 ml), then $POCl_3$ (2 ml) was added, and the mixture was refluxed for 1 h. After usual work-up, the brown residue was chromatographed on silica gel (20 g). Elution of the *Rf* 0.22 fraction (developed with benzene) with benzene gave a solid (70 mg) which was recrystallized from MeOH to give colorless platelets of **7b**, mp 152—154°C (lit.[2] 153.5—155.5°C). *Anal.* Calcd for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.20; H, 10.65. MS *m/z*: 526 (M^+), 511, 466, 451, 391, 109 (base peak), 69, 43. PMR δ (ppm): 0.69 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.61 (3H, s, 26- or 27-CH_3), 1.67 (3H, s, 26- or 27-CH_3), 2.00 (3H, s, 22-$OCOCH_3$), 2.03 (3H, s, 3-$OCOCH_3$), 4.36—4.60 (1H, m, 3-H), 4.80—5.20 (2H, m, 22- and 24-H). $[\alpha]_D^{17.5}$ +47° (c=1.0, $CHCl_3$) (lit.[2] $[\alpha]_D^{17}$ +45°).

Further elution of the *Rf* 0.11 fraction with benzene gave a solid (5 mg) which was recrystallized from MeOH to give colorless needles of **8b**, mp 143—146°C. *Anal.* Calcd for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.41; H, 10.19. MS *m/z*: 526 (M^+), 511, 466, 451 (M^+-75, base peak), 391, 109, 69, 43. PMR δ (ppm): 0.68 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.62 (3H, s, 26- or 27-CH_3), 1.68 (3H, s, 26- or 27-CH_3), 2.01 (3H, s, 22-$OCOCH_3$), 2.03 (3H, s, 3-$OCOCH_3$), 4.40—4.64 (1H, m, 3-H), 4.86—5.16 (2H, m, 22- and 24-H).

(22*R*)-22-Hydroxylanosterol (7a)——**7b** was hydrolyzed with 10% methanolic KOH under reflux for 10 h. After usual work-up, the residue was recrystallized from MeOH to give colorless needles of **7a**, mp 188—190°C (lit.[2] mp 189—191.5°C). *Anal.* Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.06; H, 11.48. MS *m/z*: 442 (M^+), 427, 409, 391, 372, 109, 99 (base peak), 69. PMR δ (ppm): 0.72 (3H, s, 18-CH_3), 0.81 (3H, s, 31-CH_3), 0.88 (3H, s, 32-CH_3), 0.98 (3H, s, 30-CH_3), 0.99 (3H, s, 19-CH_3), 1.65 (3H, s, 26- or 27-CH_3), 1.73 (3H, s, 26- or 27-CH_3), 3.12—3.36 (1H, m, 3-H), 3.56—3.80 (1H, m, 22-H), 5.08—5.32 (1H, m, 24-H). $[\alpha]_D^{17.5}$ +58° (c=0.5, $CHCl_3$) (lit.[2] $[\alpha]_D^{20}$ +59°).

(22*S*)-22-Hydroxylanosterol (8a)——**8b** was hydrolyzed with 10% methanolic KOH under reflux for 10 h. After usual work-up, the residue was recrystallized from MeOH to give colorless needles of **8a**, mp 132—134°C. *Anal.* Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 80.94; H, 11.42. MS *m/z*: 442 (M^+), 427, 409, 391, 372, 109, 99, 69 (base peak). PMR δ (ppm): 0.70 (3H, s, 18-CH_3), 0.81 (3H, s, 31-CH_3), 0.91 (3H, s, 32-CH_3), 0.98 (3H, s, 30-CH_3), 0.99 (3H, s, 19-CH_3), 1.64 (3H, s, 26- or 27-CH_3), 1.72 (3H, s, 26- or 27-CH_3), 3.12—3.36 (1H, m, 3-H), 3.56—3.80 (1H, m, 22-H), 5.08—5.32 (1H, m, 24-H). $[\alpha]_D^{17.5}$ +44° (c=0.5, $CHCl_3$).

Oxidation of 7a——Jones reagent (0.1 ml) was added to a solution of **7a** (10 mg) in acetone (5 ml), and the mixture was stirred for 15 min at 0°C. After usual work-up, the residue was chromatographed on silica gel (5 g). Elution with methylene chloride gave a solid (5 mg) which was recrystallized from MeOH to give colorless needles of lanosta-8,24-diene-3,22-dione (**9**), mp 84—85°C. *Anal.* Calcd for $C_{30}H_{46}O_2$: C, 82.13; H, 10.57. Found: C, 81.91; H, 10.45. MS *m/z*: 438 (M^+), 423, 369, 355, 341, 69 (base peak), 57, 55. PMR δ (ppm): 0.73 (3H, s, 18-CH_3), 0.92 (3H, s, 32-CH_3), 1.06, 1.08, and 1.11 (3H each, singlet each, 19-, 30- or 31-CH_3), 1.63 (3H, s, 26- or 27-CH_3), 1.76 (3H, s, 26- or 27-CH_3), 3.04—3.22 (2H, br d, 23-H_2, J=7 Hz), 5.20—5.44 (1H, m, 24-H).

Reduction of 9——$NaBH_4$ (10 mg) was added to a solution of **9** (10 mg) in MeOH (5 ml) and the reaction mixture was stirred for 1 h at room temperature. After usual work-up, the residue was chromatographed on silica gel (1 g). Elution with methylene chloride gave a solid (8 mg) which was analyzed by GLC and exhibited two peaks corresponding to **7a** and **8a** in a ratio of 1: 2. A part of the residue (6 mg) was acetylated as usual and the products were separated by chromatography on silica gel to give **7b** (2 mg) and **8b** (4 mg).

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