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Masayuki Sakakibara[a] & Kenji Mori[a]

[a] Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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Facile Synthesis of (22*R*,23*R*)-Homobrassinolide†

Masayuki SAKAKIBARA and Kenji MORI

Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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A synthesis of (22*R*,23*R*)-homobrassinolide is described. The LC and the chemical correlation studies for the oxidation product of a stigmasterol-like side chain with osmium tetroxide are mentioned. A stereochemical view for the mechanism of osmium tetroxide oxidation of the side chain is proposed.

Brassinolide **1a**, isolated from the pollen of rape (*Brassica napus* L.), is a steroidal B-homolactone with plant-growth promoting activity.[1] The structure, including the stereochemistry, was established by X-ray crystallographic analysis.[2] Total synthesis of brassinolide has been achieved by three groups[3～5,11] so far, and the synthetic works on its analogs have been reported by several groups.[6～9] In the results, it was pointed out that the presence of some structural moieties, including the stereochemistry, is important for high potency in bioassays.[3,6,8～10] We now report the facile synthesis of (22*R*,23*R*)-homobrassinolide **1b** which possesses the same stereochemistry as brassinolide **1a** itself but with an ethyl group instead of a methyl group at the 24-position in **1a**.

In an earlier work[7] by one of the authors, the problems of stereoselectivity in osmium tetroxide oxidation and the stereochemistry at 22- and 23-positions of the product **4a** remained unresolved. In order to solve these problems, several attempts were made. The LC analysis on a less polar diacetonide derivative **2a** than the original oxidation product **4a** revealed extremely high stereoselectivity in the oxidation reaction. Namely, a crystalline material obtained from the acetonide formation of a crude oxidation[32] product of (22*E*,24*S*)-24-ethyl-5α-cholesta-2,22-dien-6-one[7] showed three peaks on a liquid chromatograph. The most intense peak was assigned to **2a** which was obtained in a pure form from the oxidation[31] product **4a** of pure (2*R*,3*S*,22*E*,24*S*)-24-ethyl-2,3-dihydroxy-5α-cholest-22-en-6-one.[5] However, neither of the residual two peaks was assigned to **2b**,*1a (22*R*,23*R*)-isomer of **2a**. The stereoisomeric relationship of **2a** and **2b** was verified by the identity of a mass spectrum of **2a** with that of **2b**. Under the tested condition during the LC analysis, **2b** is less polar than **2a**. Consequently, the oxidation reaction with osmium tetroxide proceeded in a virtually stereospecific manner in our case. However, poor yields of the (22*R*,23*R*)-isomers were reported in other cases lacking a 2-functional group.[9]

The acetonide **2a** was deduced to possess the (22*S*,23*S*)-stereochemistry from a comparative study of the CMR data of **4a**, **4b** and natural brassinolide **1a**, as well as from the result of our chemical correlation study described later and the result of the CMR study by Wada and Marumo.[8] This deduction was finally confirmed by the X-ray crystallographic analysis of **2a** as reported separately.[11]

In order to obtain the desired (22*R*,23*R*)-stereochemistry, several attempts were made. Haloacyloxylation of the enone **3** in a couple of conditions such as iodine/potassium iodate

† Brassinolide and Its Analogs. Part II. For Part I, see ref. 11.

*1 For the preparation of **2b** and **4b**, see EXPERIMENTAL.

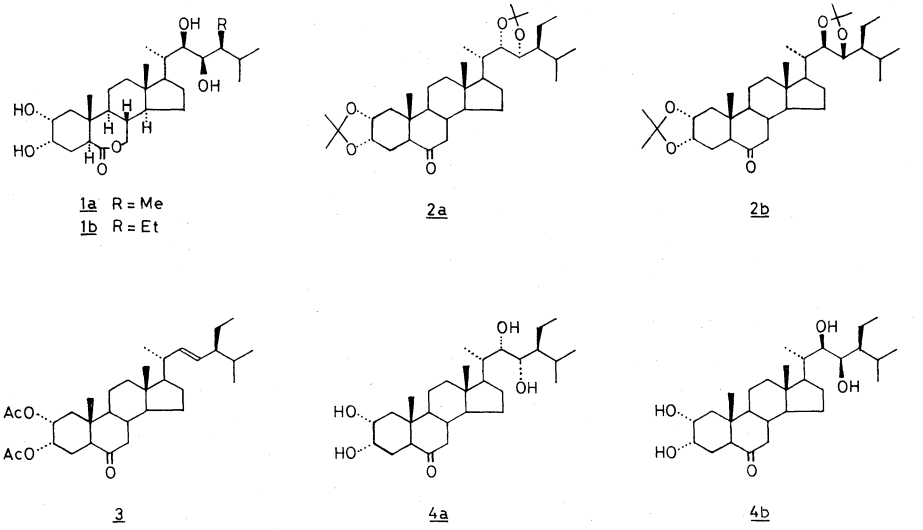


FIG. 1.

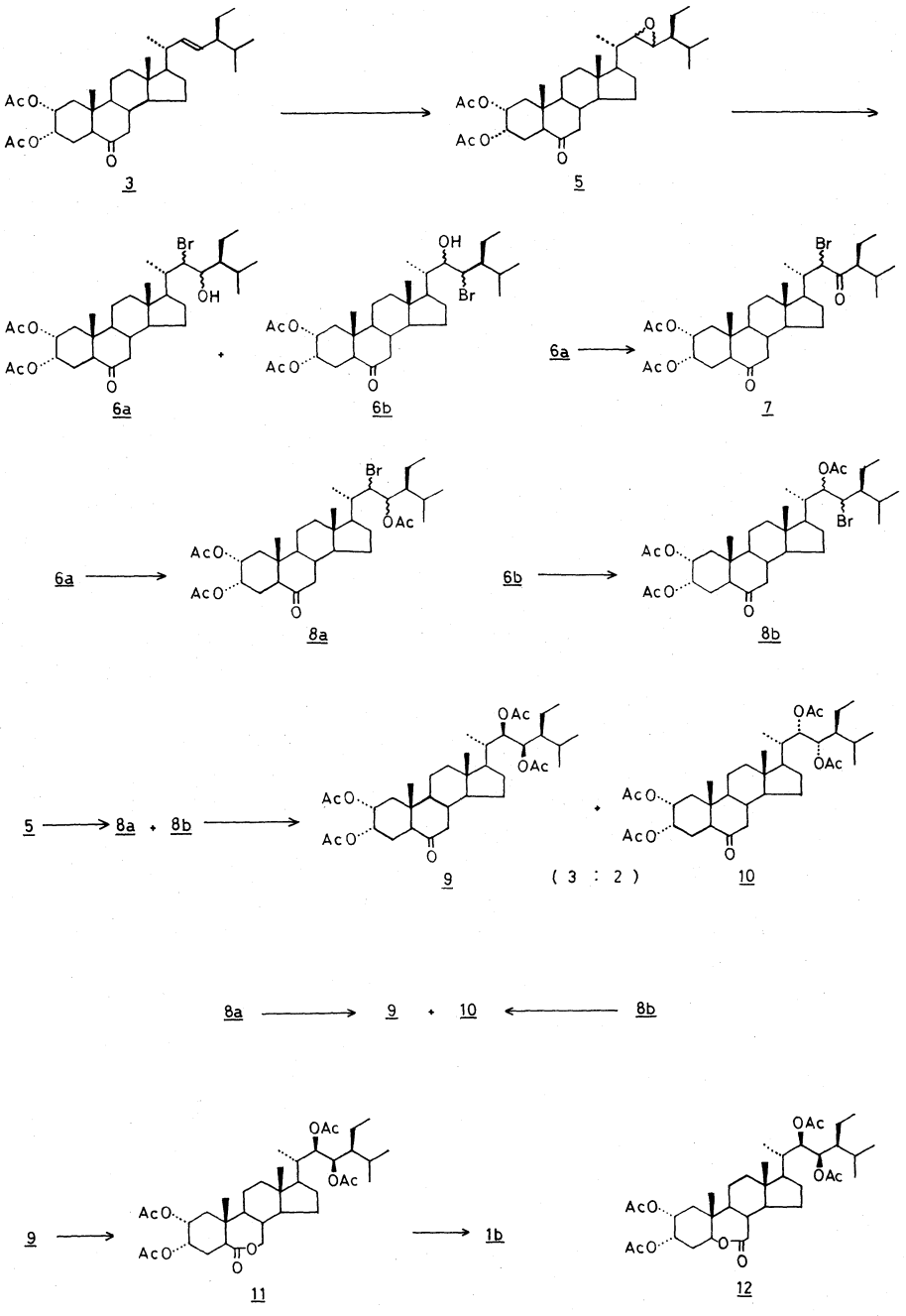
in acetic acid,[12] *N*-iodosuccinimide/formic acid in chlorform,[13] iodine triacetate,[14] and *N*-bromosuccinimide/silver acetate in acetic acid,[15] followed by acid hydrolysis,[15,30] gave extremely poor results.

Epoxidation of **3** with *m*-chloroperbenzoic acid[16] gave an inseparable diastereoisomeric mixture of the epoxides **5** in 93.9% yield. To learn the major product, the mixture was treated with 48% hydrobromic acid.[16] Chromatographic separation of the resulting regioisomeric mixture of bromohydrins gave a less polar product **6a** in 76.7% yield and a more polar product **6b** in 21.6% yield, which were diastereoisomeric mixtures. The structures of **6a** and **6b** were deduced from a comparison of their mass spectra. Additionally, a mass spectrum of a Jones oxidation product **7** from the less polar **6a** supported the structure of **6a** possessing a 22-bromo-23-hydroxyl system; namely, **6a** was oxidized with Jones reagent[33] to give a bromodiketone **7** in 76.5% yield, whose mass spectrum showed a very intense peak at *m/z* 113 corresponding to $C_7H_{13}O$ ($Me_2CHEtCHCO^+$). A CD spectrum of **7** showed a negative Cotton effect ($[\theta]_{334.5\,nm}$ −10760) which suggested that a desired (22*S*)-bromo isomer was the major product.[16]

6a was next acetylated with acetic anhydride in pyridine, containing a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine,[34] to give a triacetoxybromoketone **8a** in 90.8% yield. Hydrolysis of **8a** with 80% aqueous acetic acid at 90°C,[15,30] followed by acetylation, gave a mixture of (22*R*,23*R*)- and (22*S*,23*S*)-tetraacetates **9** and **10**. Chromatographic separation of the mixture gave a less polar **10**[7] in 30.7% yield and a more polar **9** in 47.1% yield in pure forms. The more polar bromohydrin **6b** was also treated as described above to give **9** in 86% yield and **10** in 14% yeild. The tetraacetates **9** and **10** were also obtained by epoxide ring-opening of **5** with 30% hydrobromic acid in acetic acid followed by successive manipulations as mentioned above in 42.7% and 27.0% yields, respectively.

Baeyer-Villiger oxidation of **9** with trifluoroperacetic acid in the presence of disodium hydrogen phosphate in dichloromethane,[5,7] followed by chromatographic purification gave a tetraacetoxylactone **11** (mp 137~139°C; $[\alpha]_D^{24}$ +36.4° (*c* 0.662, $CHCl_3$)) in 78.6% yield, along with a mixture (20% yield) of **11** and its likely regioisomer **12**.*[2] The lactone **11** was hydrolyzed with sodium hy-

*[2] No evidence; an attempt for purification was unsuccessful.



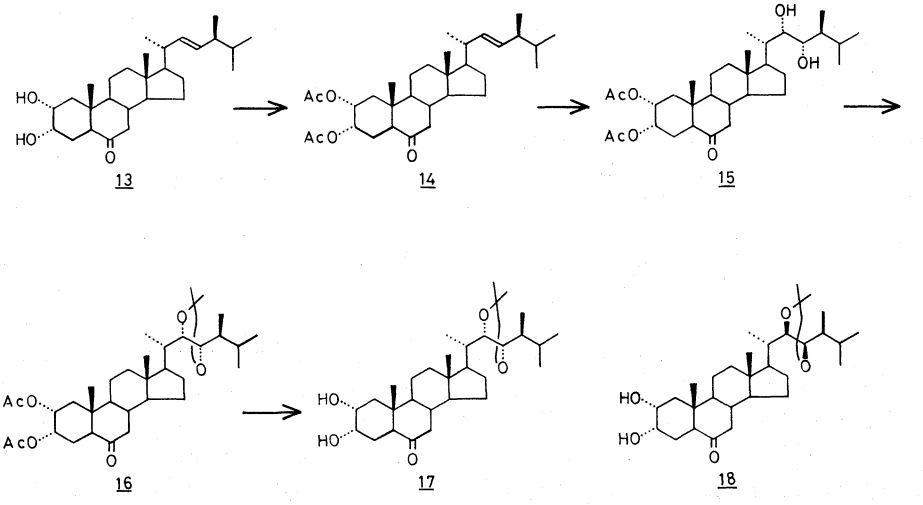
droxide in aqueous methanol and relactonized with 6 N hydrochloric acid to give (22*R*,23*R*)-homobrassinolide **1b** (mp 249~251°C; $[\alpha]_D^{24}$ +42.9° (*c* 0.681, $CHCl_3$–CH_3OH (9 : 1))) in 61.3% yield after recrystallization from methanol. **1b** was shown to possess much more potency than (22*S*,23*S*)-homobrassinolide[7] and a comparable, or slightly less, potency to brassinolide **1a** itself in the lamina-joint test.[35]

As additional supporting evidence for the stereochemistry of **4a**, we carried out a chemical correlation study employing an intermediate in our brassinolide synthesis.[5,11]

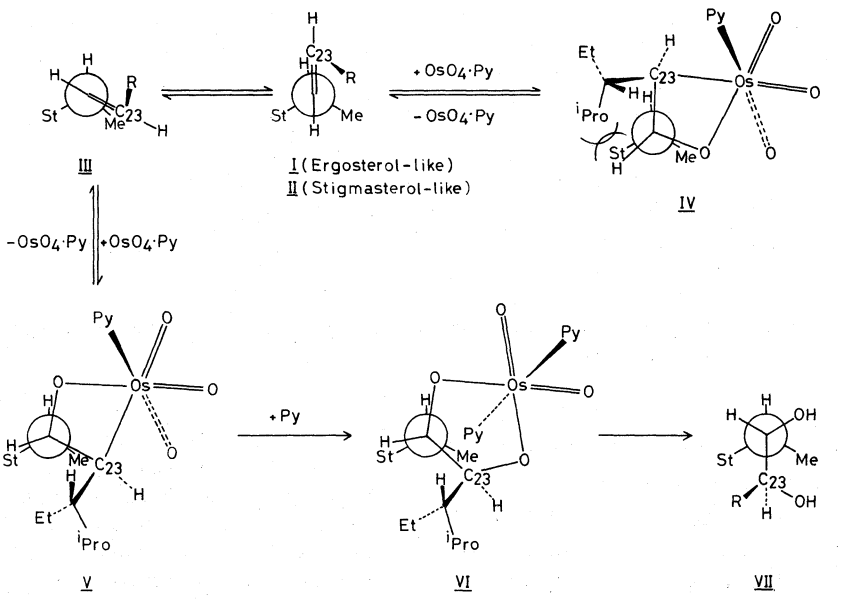
(2*R*,3*S*,22*E*,24*S*)-2,3-Dihydroxy-24-methyl-5α-cholest-22-en-6-one **13** was acetylated to give a diacetate **14** in 95.3% yield. The oxidation of **14** with osmium tetroxide in pyridine,[31] followed by the acetonide formation, gave an acetonide **16** *via* a tetraol diacetate **15**. **16** was hydrolyzed in the usual manner to afford a tetraol monoacetonide **17**. The melting point of **17** (mp 98～100°C) was definitely different from that of Siddall's compound **18** (mp 216～218°C).[3] This fact led us to deduce the (22*S*,23*S*)-stereochemistry of the oxidation product from stigmasterol-like sterols with osmium tetroxide.

Finally, we propose the following mechanism on osmium tetroxide oxidation of the stigmasterol-like side chain, producing a (22*S*,23*S*)-diol system in a highly stereoselective or a virtually stereospecific manner. Barton *et al.* revealed the conformation of the ergosterol-like side chain in the ground state by X-ray crystallographic analysis of ergocalciferol, as depicted in Formula **I**.[17] Additionally, the restricted rotation around the C(20)–C(22) bond has been mentioned even in solution.[16] However, it does no more than imply the presence of the most stable conformation. The most stable conformation is undoubtedly important in the reaction involving a reagent of such a small effective molecular size as *m*-chloroperbenzoic acid (0.09 Å of an ion radius for O^+),*3 because such a small reagent would not be sterically affected by a substituent at the 20-position and could approach the reaction point through a course within a comparatively wide scope on the other side of a steroidal nucleus. In such a type of reaction, the stereochemical outcome of the reaction will be controlled by the conformation as reported in refs. 5, 11, 16, 18 and in the present paper.

Contrary to this, a reagent of such a large effective molecular size as an alkyl Grignard reagent (at least *ca.* 1.1 Å of an anion radius for Me^-), iodine (0.50 Å of an ion radius for I^+), or osmium tetroxide (2.01 Å of a bond length for Os=O) would approach the reaction point in a somehow restricted manner because of steric hindrance by the 20-methyl group and even a 20-hydrogen atom; namely, only an approach from the other direction of a steroidal nucleus would be permitted.[16,18,20,21] Since only the conformer(s) coincident with the direction of the approach of the reagent would participate in a reaction, the most stable conformation would not necessarily reflect on the stereochemical outcome of the reaction. To simplify the following discussion on the mech-



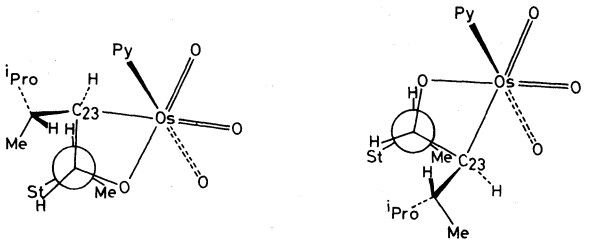
*3 A periodic table referring to the observed values by Wyckoff (1948) and the calculated values by L. Pauling (1927).



anism of osmium tetroxide oxidation, therefore, we would like to consider only two restricted conformers **II** and **III**, fit for the approach of the oxidant.

Additionally, the outcome of the reaction would be governed also by the reaction course; namely, the involvement or lack of a reversible process, the behavior of an intermediate in the rate-determining step, the coordination of a counterion with a heteroatom prior to a nucleophilic addition, *etc.* For osmium tetroxide oxidation, the initial formation of a three-membered ring organometallic intermediate and rearrangement in the rate-determining step were suggested earlier on the basis of the thermodynamic study[26] as reviewed.[27] Recently, the presence of the reversible process and the coordination of the second ligand molecule with the intermediate complex in the rate-determining step were concluded on the basis of the kinetic studies[22~24] as reviewed.[27] More recently, the mechanism involving the initial coordination of a weakly basic olefin with the oxidant, followed by the formation of a four-membered ring organometallic intermediate, rearrangement and the additional coordination by a ligand molecule to give a well-known five-membered ring intermediate, was proposed,[25] though the initial step was illustrated in an irreversible manner.

From these viewpoints, the probable mechanism producing a (22*S*,23*S*)-diol from the stigmasterol-like side chain is as follows. The incorporation of an osmium tetroxide–pyridine complex (OsO_4 : Py) into the conformer **II** might form the four-membered ring organometallic intermediate **IV**. However, as the sp^3 character at the 22- and 23-carbon atoms enhances, a bulky isopropyl group orientated to the steroidal nucleus should give rise to drastic steric hindrance with a 16-methylene group in such a conformation as a 24-hydrogen atom orientates *syn*-likewise to a huge osmium atom. Consequently, the reaction would proceed to the liberation of OsO_4 : Py from **IV** and the reproduction of the substrate. While the incorporation of OsO_4 : Py into the conformer **III** would result in the intermediate **V** and this would be much more preferred than **IV** because a less bulky ethyl group would be located in the less hindered back side of the steroidal nucleus and a bulkier isopropyl group would be remote from it. In this case, the coordination of one more pyridine molecule with **V**, accompanied by simultaneous rearrangement in the rate-determining step, would result in a stable five-membered cyclic ester complex **VI** which



VIII IX

FIG. 2. Conformational Views of Two Regioisomeric Organoosmium Intermediates Derived from an Ergosterol-like Side Chain.

would be reductively or oxidatively hydrolyzed to give a (22*S*,23*S*)-diol **VII**.

Our speculation on the mechanism could account for the result of the USDA group[6] that both (22*R*,23*R*)- and (22*S*,23*S*)-diols were obtained by osmium tetroxide oxidation of thier olefin with a (24*R*)-methyl group. Namely, both organometallic intermediates **VIII** and **IX** would almost equally survive in the case of an ergosterol-like side chain possessing the (24*R*)-methyl group, because a less bulky methyl group would be settled at the more hindered upperside of the steroidal nucleus in **VIII** and a bulkier isopropyl group would be likewise at the less hindered back side of it in **IX**. The outcome of the USDA group would be a reflection by the co-existance of **VIII** and **IX**.

Our speculation has no dissonance with that of the mechanism for the nucleophilic addition of alkyl Grignard reagents to a 22-formyl group[17,28~30] and also with that of the mechanism for halohydrinations and haloacyloxylations[18] of a 22-olefin.

EXPERIMENTAL

All melting points were uncorrected. IR spectra were determined on a Jasco A-102 spectrometer, PMR spectra were recorded on a Hitachi R-24A, a Jeol JNM-MH-100, or a Jeol JNM-FX-400 NMR spectrometer. CMR spectra were recorded on a Jeol JNM-FX-100 NMR spectrometer. Chemical shifts are expressed in ppm downfield from TMS as an internal standard. Mass spectra were measured on a Hitachi RM-61 mass spectrometer. The CD spectrum was recorded on a Jasco J-20 ORD-CD spectrometer. Specific rotations were determined on a Jasco DIP-4 or a Jasco DIP-140 digital polarimeter. Liquid chromatography was carried out on a Shimadzu LC-2 liquid chromatograph, using a Senshu SC-4.6 column (4.6 mm i.d. × 6.35 mm o.d. × 250 mm) packed with Whatman Partisil-5 (5 μm silica gel) as the medium and a Shimadzu RID-4 refractometer as a detector. The elution system used was *n*-hexane–ethyl acetate (5 : 1) and the flow rate was 2.0 ml/min. Merck Art. 7734 Kieselgel 60 (70 ~ 230 mesh) or Mallinckrodt Silicar CC-7 was used for column chromatography. Merck Art. 5714 Kieselgel 60F_{254} (layer thickness 0.25 mm) was used for TLC and Merck Art. 5717 Kieselgel 60F_{254} (layer thickness 2 mm) was used for preparative layer chromatography.

(2R,3S,24S)-2,3-Diacetoxy-22,23-epoxy-24-ethyl-5α-cholestan-6-one **5**. *m*-Chloroperbenzoic acid (1889 mg; 10.95 mmol) was added to a stirred solution of the diacetoxyenone **3** (2313 mg; 4.38 mmol) in dichloromethane (100 ml) on an ice bath. After 30 min, the ice bath was removed and stirring was continued for 5 hr at room temperature. The reaction mixture was washed with 1 N sodium hydroxide (100 ml) and then with water (100 ml), dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give a colorless and syrupy residue, which was crystallized from 99% ethanol to give colorless needles of **5** (2238 mg; 93.9% yield); mp 156 ~ 159°C; $[\alpha]_D^{24}$ −9.16° (*c* 0.982, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 1750 (s), 1740 (sh), 1705 (s), 1460 (m), 1440 (sh), 1380 (sh), 1370 (m), 1250 (s), 1230 (m), 1170 (w), 1150 (w), 1120 (w), 1090 (w), 1040 (m), 1025 (sh), 990 (w), 955 (w), 930 (w), 900 (m), 870 (w). PMR (60 MHz; $CDCl_3$) δ: 0.66 (s, 3H), 0.75 ~ 2.85 (m), 1.95 (s, 3H), 2.04 (s, 3H), 2.5 (m, 2H), 4.65 ~ 5.15 (m, 1H), 5.20 ~ 5.45 (m, 1H). CMR (25 MHz; $CDCl_3$) δ: 210.2, 170.1, 169.8, 69.1, 68.1. MS (*m/z*): 544 (M^+), 484 (M^+–AcOH), 459 ($M^+ - C_6H_{13}$). *Anal.* Found; C: 72.77, H: 9.65. Calcd. for $C_{33}H_{52}O_6$; C: 72.75, H: 9.62.

(2R,3S,22R,23R,24S)-2,3,22,23-Tetraacetoxy-24-ethyl-5α-cholestan-6-one **9**. A mixture of the epoxide **5** (1013 mg; 2 mmol) and 30% hydrogen bromide in acetic acid (4 ml) was stirred for 3 hr at room temperature, followed by the addition of water (40 ml) and neutraliza-

tion with solid sodium carbonate. The mixture was extracted three times with ether. The ethereal extracts were combined, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a gummy mixture (1.3 g) of **8a** and **8b**.

A mixture of the residue (1.3 g) obtained, glacial acetic acid (40 ml) and water (10 ml) was stirred for 15.5 hr at 90～100°C. The reaction mixture was poured into chilled aqueous sodium hydrogen carbonate. This mixture was extracted three times with ethyl acetate. The extracts were combined, washed three times with aqueous sodium hydrogen carbonate and once with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a gummy residue.

To a solution of the gummy residue in dry pyridine (20 ml), acetic anhydride (20 ml) and 4-(*N*,*N*-dimethylamino)pyridine (50 mg) were added and the mixture was stirred for 20 hr at room temperature. The reaction mixture was poured into dilute hydrochloric acid and the mixture was extracted twice with ether. The ethereal extracts were combined, successively washed twice with dilute hydrochloric acid, once with water, five times with aqueous sodium hydrogen carbonate, and once with brine, dried over anhydrous sodium sulfate, and finally evaporated under reduced pressure to give a gummy substance (1.3 g). Column chromatography with silica gel (Merck; 78 g), which was eluted with 15% ethyl acetate in *n*-hexane, gave an initial recovery of **8a** and **8b** (218 mg; 17.6% yield), followed by an undesired (22*S*,23*S*)-isomer **10** (325 mg; 27.0% yield): $[\alpha]_D^{23}$ −14.8° (*c* 0.715, $CHCl_3$); PMR (100 MHz; CCl_4) δ: 0.68 (s, 3H), 0.8～2.8 (m), 1.96 (s, 3H), 2.04 (s, 6H), 2.08 (s, 3H), 4.8～5.1 (m, 2H), 5.1～5.5 (m, 2H), and finally, the desired product **9** (514 mg; 42.7% yield) as a gummy substance: $[\alpha]_D^{23}$ +3.60° (*c* 0.639, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 1740 (s), 1710 (s), 1460 (m), 1370 (m), 1240 (s), 1220 (s), 1170 (w), 1150 (w), 1105 (w), 1070 (w), 1040 (m), 1020 (m), 985 (w), 978 (w), 945 (w), 900 (w), 880 (w), 855 (w). PMR (100 MHz; CCl_4) δ: 0.71 (s, 3H), 0.76～2.8 (m), 1.92 (s, 6H), 1.98 (s, 3H), 2.02 (s, 3H), 4.7～5.0 (m, 1H), 5.0～5.4 (m, 3H). MS (*m/z*): 646 (M^+), 586 (M^+−AcOH), 526 (M^+−2AcOH), 501 (M^+−AcOH−C_6H_{13}).

The tetraacetates **9** and **10** were also obtained from **8a** and **8b** in the same manner as described in the direct preparation from **5**; namely, **9** (47.1% yield) and **10** (30.7% yield) from **8a**, and **9** (86.0% yield) and **10** (14.0% yield) from **8b**.

(2R,3S,22R,23R,24S)-2,3,22,23-Tetraacetoxy-24-ethyl-B-homo-7-oxa-5α-cholestan-6-one **11**. A peracid solution prepared from 90% hydrogen peroxide (273 μl), trifluoroacetic anhydride (1781 μl), and dry dichloromethane (2.7 ml), was added dropwise to a stirred mixture of the tetraacetoxy ketone **9** (323 mg; 0.5 mmol), finely pulverized disodium hydrogen phosphate (1443 mg), and dry dichloromethane (15 ml) on an ice bath. The ice bath was removed and the reaction mixture was stirred for 30 min at room temperature and then for 1 hr at reflux temperature. After cooling, iced water was added to the reaction mixture and a dichloromethane layer was separated. An aqueous layer was extracted with dichloromethane. The organic solutions were combined, washed with aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a gummy substance (362 mg), which was purified by column chromatography with silica gel (Merck; 21 g), which was eluted with 20～30% ethyl acetate in *n*-hexane, to give a colorless syrupy substance (260 mg; 78.5% yield). Crystallization from 99% ethanol gave colorless crystals (240 mg); recrystallization from 99% ethanol gave colorless plates (mp 137～139°C) of **11**; $[\alpha]_D^{24}$ +36.4° (*c* 0.662, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 1740 (s), 1720 (sh), 1460 (m), 1370 (m), 1330 (w), 1305 (w), 1248 (s), 1223 (s), 1182 (w), 1170 (w), 1135 (w), 1120 (sh), 1075 (w), 1060 (sh), 1050 (m), 1020 (m), 995 (sh), 980 (w), 942 (w), 915 (w). PMR (100 MHz; CCl_4) δ: 0.76 (s, 3H), 0.8～2.6 (m), 1.91 (s, 3H), 1.93 (s, 3H), 1.95 (s, 3H), 2.04 (s, 3H), 2.93 (m, 1H), 4.02 (m, 2H), 4.6～5.0 (m, 1H), 5.0～5.4 (m, 3H). MS (*m/z*): 662 (M^+), 602 (M^+−AcOH), 578 (M^+−EtCH=CMe_2), 560 (M^+−AcOH−CH_2=CO), 549 (M^+−Me_2CHCHEtCO), 548 (M^+−Me_2CHCHEtCHO), 542 (M^+−2AcOH), 517 (M^+−AcOH−Me_2CHCHEt), 506 (M^+−MeCO−Me_2CHCHEtCO), 463 (*m/z* 506-MeCO), 403 (*m/z* 463-AcOH), 360 (*m/z* 403-MeCO), 343 (*m/z* 403-AcOH). *Anal.* Found; C, 66.59, H: 8.73. Calcd. for $C_{37}H_{58}O_{10}$; C: 67.04, H: 8.82.

(2R,3S,22R,23R,24S)-24-Ethyl-2,3,22,23-tetrahydroxy-B-homo-7-oxa-5α-cholestan-6-one((22R,23R,)-homobrassinolide) **1b**. A solution of sodium hydroxide (480 mg) in water (1.2 ml) was added to a solution of **11** (232 mg) in methanol (20 ml). The mixture was stirred for 3 hr at reflux temperature and for 40 hr at room temperature.

Tetrahydrofuran (20 ml) was added to the reaction mixture and the mixture was acidified with 6 N hydrochloric acid (6 ml), stirred for 4 hr at room temperature and evaporated under reduced pressure. The residue obtained was neutralized with solid sodium hydrogen carbonate and extracted twice with chloroform. The extracts were combined, dried over anhydrous sodium sulfate and evaporated under reduced pressure to give colorless crystals (180 mg) which were recrystallized from methanol to give colorless fine needles (106 mg; 61.3% yield) of **1b**; mp 249～251°C; $[\alpha]_D^{24}$ +42.9° (*c* 0.681, $CHCl_3$–MeOH, 9:1). ν_{max}^{nujol} (cm^{-1}): 3450 (s), 1730 (s), 1720 (sh), 1695 (s), 1460 (s), 1403 (m), 1377 (s), 1330 (m), 1318 (m), 1290 (w), 1285 (m), 1255 (w), 1220 (w), 1180 (m), 1160 (w), 1140 (w), 1120 (w), 1102 (w), 1060 (s), 1020 (s), 980 (m), 930 (w), 915 (w), 875 (w), 860 (w), 790 (w), 782 (w), 735 (w), 710 (w), 700 (w), 660 (w). PMR (400.5 MHz; C_5D_5N) δ: 0.73 (s, 3H), 1.05 (s, 3H), 1.07 (d, J=6.8 Hz, 3H), 1.10 (t, J=7.6 Hz, 3H), 1.15 (d, J=6.8 Hz, 3H), 1.21 (d, J=6.8 Hz, 3H),

1.25~2.27 (m), 2.31 (dt, J=4.4 and 14.6 Hz, 1H), 2.51 (ddd, J=2.0, 12.2 and 14.6 Hz, 1H), 3.61 (dd, J=4.4 and 12.2 Hz, 1H), 3.97 (d, J=8.8 Hz, 1H), 4.00~4.14 (m, 4H), 4.43 (br. s, 1H). CMR (25 MHz; $CDCl_3$–CD_3OD, 4:1) δ: 178.2, 74.6, 72.8, 71.1, 68.2, 68.2, 58.5, 52.9. 42.9, 41.4, 40.1, 39.6, 38.6, 37.6, 31.7, 29.5, 27.8, 25.1, 22.6, 21.4, 19.7, 19.5, 19.3, 15.6, 13.9, 12.1, 11.9. *Anal.* Found; C, 70.23, H: 10.20. Calcd. for $C_{29}H_{50}O_6$; C: 70.41, H: 10.19.

(2R,3S,22E,24S)-2,3-Diacetoxy-24-methyl-5α-cholest-22-en-6-one **14**. This was prepared as described in refs. 5) and 11).

(2R,3S,22S,23S,24S)-2,3-Diacetoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one **15**. A solution of osmium tetroxide (7 mg; 0.027 mmol) in dry pyridine (56 μl) was added to a stirred solution of **14** (11 mg; 0.02 mmol) in dry pyridine (1 ml) under an argon atmosphere and the mixture was stirred for 10 days in darkness. A solution of sodium hydrogen sulfite (120 mg) in a mixture of water (1.9 ml) and pyridine (1.3 ml) was added to the reaction mixture and the stirring was continued for 30 min. The mixture was extracted with three portions of chloroform. The combined extracts were successively washed twice with water, three times with dilute hydrochloric acid, once with water and once with aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give a crystalline residue, recrystallization of which from 99% ethanol gave colorless needles (11 mg) of **15**; mp 226~228°C. ν_{max}^{nujol} (cm^{-1}): 3450 (s), 3380 (s), 3300 (s), 1740 (s), 1710 (sh), 1704 (s), 1675 (w), 1459 (s), 1383 (s), 1370 (s), 1323 (w), 1306 (w), 1261 (s), 1231 (s), 1215 (m), 1150 (w), 1118 (w), 1099 (w), 1080 (m), 1058 (m), 1038 (s), 1020 (s), 992 (m), 981 (w), 960 (w), 950 (w), 938 (w), 900 (w), 862 (w), 830 (w). PMR (400 MHz; $CDCl_3$) δ: 0.71 (s, 3H), 0.75 (d, J=7.1 Hz, 3H), 0.81 (d, J=6.8 Hz, 3H), 0.83 (s, 3H), 0.94 (d, J=6.8 Hz, 3H), 1.05 (d, J=6.8 Hz, 3H), 1.99 (s, 3H), 2.09 (s, 3H), 1.09~2.19 (m), 2.34 (dd, J=4.5 and 13.3 Hz, 1H), 2.57 (dd, J=4.3 and 11.6 Hz, 1H), 3.46 (dd, J=7.3 and 9.0 Hz, 1H), 3.68 (dd, J=4.5 and 6.3 Hz, 1H), 4.95 (ddd, J=3.2, 4.9 and 12.2 Hz, 1H), 5.39 (br. d, J=3.2 Hz, 1H). MS (m/z): 530 (M^+-H_2O), 488 (M^+–AcOH), 470 (M^+-H_2O–AcOH), 459 ($M^+-H_2O-C_5H_{11}$), 447, 429, 388 (M^+–AcOH–$C_5H_{11}CHO$), 375, 358 (M^+–AcOH–$C_5H_{11}CHOHCHO$), 346 (M^+–AcOH–$C_5H_{11}CHO$–CH_2=CO), 345 (M^+–AcOH–$C_5H_{11}CHO$–MeCO), 328 (M^+–AcOH–$C_9H_{20}O_2$), 327 (M^+–AcOH–$C_5H_{11}CHO$–MeCO–H_2O), 316 (M^+–AcOH–$C_5H_{11}CHO$–CH_2=CO–HCHO).

(2R,3S,22S,23S,24S)-2,3-Diacetoxy-22,23-isopropylidenedioxy-24-methyl-5α-cholestan-6-one **16**. A solution of osmium tetroxide (52 mg) and **14** (95 mg; 0.185 mmol) in dry pyridine (1 ml) was stirred in darkness for 24 hr at room temperature under an argon atmosphere. A solution of sodium hydrogen sulfite (120 mg) in a mixture of water (1.9 ml) and pyridine (1.3 ml) was added to the reaction mixture and the stirring was continued for 30 min. The mixture was poured into water and extracted with three portions of chloroform. The combined extracts were successively washed twice with water, three times with dilute hydrochloric acid, once with water and once with aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate and evaporated under reduced presssure to give a crystalline residue (120 mg).

A solution of the residue obtained (110 mg), 2,2-dimethoxypropane (1 ml) and a catalytic amount of *p*-toluenesulfonic acid in dry dichloromethane (2 ml) was stirred for 17 hr at room temperature, washed once with aqueous sodium hydrogen carbonate, dried over anhydrous potassium carbonate and evaporated under reduced pressure to give a crystalline residue. Chromatographic purification with silica gel (Merck: 4 g) which was eluted with 2.5~10% ethyl acetate in *n*-hexane gave the desired product **16** (19 mg) along with the unreacted **14** (11 mg). Recrystallization of **16** from 99% ethanol gave colorless needles, mp 158~159°C. ν_{max}^{nujol} (cm^{-1}): 1760 (s), 1741 (s), 1715 (s), 1460 (m), 1419 (w), 1398 (w), 1375 (m), 1367 (m), 1300 (w), 1242 (s), 1223 (s), 1195 (w), 1110 (w), 1045 (s), 1018 (w), 990 (w), 900 (w). PMR (400 MHz; $CDCl_3$) δ: 0.71 (s, 3H), 0.80 (d, J=7.1 Hz, 3H), 0.83 (s, 3H), 0.85 (d, J=6.8 Hz, 3H), 0.92 (d, J=6.8 Hz, 3H), 1.03 (d, J=6.6 Hz, 3H), 1.36 (s, 3H), 1.42 (s, 3H), 1.99 (s, 3H), 2.08 (s, 3H), 1.08~2.19 (m), 2.33 (dd, J=4.5 and 13.3 Hz, 1H), 2.57 (dd, J=4.1 and 11.4 Hz, 1H), 3.72 (dd, J=6.8 and 7.1 Hz, 1H), 3.88 (dd, J=3.1 and 6.0 Hz, 1H), 4.95 (ddd, J=3.1, 4.6 and 12.5 Hz, 1H), 5.38 (br. d, J=2.9 Hz, 1H). MS (m/z): 588 (M^+), 587 (M^+-H), 573 (M^+–Me), 517 ($M^+-C_5H_{11}$), 473 (M^+–Me–$C_5H_{11}CHO$), 387, 370 (M^+–Me–AcOH–MeCO–$C_5H_{11}CHO$). *Anal.* Found; C: 71.98, H: 9.38. Calcd. for $C_{35}H_{56}O_7$; C: 71.39, H: 9.59.

(2R,3S,22S,23S,24S)-2,3-Dihydroxy-22,23-isopropylidenedioxy-24-methyl-5α-cholestan-6-one **17**. A solution of potassium hydroxide (10 mg) in aqueous methanol (2 ml of methanol and 0.2 ml of water) was added to a solution of **16** (11 mg) in methanol (3 ml). The mixture was heated with stirring at reflux temperature for 3 hr, poured into water and extracted three times with ethyl acetate. The extracts were combined, washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a gummy residue, which was crystallized from 99% ethanol to give colorless needles (8 mg) of **17**, mp 98~100°C. ν_{max}^{nujol} (cm^{-1}): 3400 (br, s), 1713 (s), 1460 (s), 1378 (s), 1368 (m), 1307 (w), 1278 (w), 1255 (m), 1240 (m), 1220 (w), 1205 (w), 1163 (m), 1120 (m), 1090 (w), 1080 (m), 1060 (m), 1045 (s), 1015 (m), 995 (w), 930 (w), 890 (w), 875 (w), 859 (w). PMR (400 MHz; $CDCl_3$) δ: 0.70 (s, 3H), 0.76 (s, 3H), 0.81 (d, J=7.1 Hz, 3H), 0.85 (d, J=6.8 Hz, 3H), 0.92 (d, J=7.1 Hz, 3H), 1.03 (d, J=6.8 Hz, 3H), 1.36 (s, 3H), 1.42 (s, 3H), 1.08~2.18 (m), 2.30 (dd, J=4.6 and 13.2 Hz, 1H), 2.68 (dd, J=2.7 and 12.7 Hz, 1H),

3.72 (dd, $J=6.3$ and 7.4 Hz, 1H), 3.77 (br. dt, $J=3.5$ and 11.0 Hz, 1H), 3.89 (dd, $J=3.2$ and 6.1 Hz, 1H), 4.05 (br. d, $J=2.7$ Hz, 1H). MS (*m/z*): 504 (M^+), 503 (M^+-H), 490, 489 (M^+-Me), 434, 433 ($M^+-C_5H_{11}$), 404 ($M^+-C_5H_{11}CHO$), 389 ($M^+-Me-C_5H_{11}CHO$).

(2R,3S,24S)-2,3-Diacetoxy-22-bromo-24-ethyl-23-hydroxy-5α-cholestan-6-one **6a** *and (2R,3S,24S)-2,3-diacetoxy-23-bromo-24-ethyl-22-hydroxy-5α-cholestan-6-one* **6b**. A mixture of acetic acid (1.5 ml) and 47% hydrobromic acid (1.5 ml) was added to a stirred solution of **5** (310 mg; 0.57 mmol) in chloroform (30 ml) and stirring was continued for 42 hr at room temperature. The reaction mixture was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crystalline residue obtained was chromatographed with silica gel (20 g). A less polar bromohydrin **6a** was obtained from the fractions eluted with ethyl acetate–*n*-hexane (15:85). Recrystallization of **6a** from 99% ethanol-dichloromethane gave fine needles (273 mg; 76.7% yield), mp 222~223°C; $[\alpha]_D^{24}$ −9.21° (*c* 0.532, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 3475 (m), 1741 (s), 1720 (s), 1703 (s), 1459 (m), 1383 (m), 1362 (m), 1301 (w), 1270 (s), 1258 (m), 1250 (m), 1230 (s), 1205 (w), 1168 (w), 1148 (w), 1121 (w), 1098 (w), 1077 (w), 1038 (m), 1020 (w), 998 (w), 980 (w), 975 (w), 930 (w), 898 (w), 858 (w), 817 (w). PMR (60 MHz, $CDCl_3$) δ: 0.70 (s, 3H), 0.73 (s, 3H), 0.8~2.8 (m), 1.97 (s, 3H), 2.07 (s, 3H), 4.05 (m, 2H), 4.90 (m, 1H), 5.33 (m, 1H). CMR (25 MHz; $CDCl_3$) δ: 210.5, 170.1, 169.9, 73.0 (76.4%), 72.6 (23.6%), 69.1, 68.1; CMR data revealed **6a** to be a diastereoisomeric mixture. MS (*m/z*): 626 and 624 (M^+), 566 and 564 (M^+-AcOH), 544 (M^+-HBr), 484 ($M^+-HBr-AcOH$), 459 ($M^+-HBr-C_6H_{13}$), 452 and 450 ($M^+-AcOH-C_6H_{13}CHO$), 417 ($M^+-C_6H_{13}CHOHCHBr$), 115 ($C_6H_{13}CHOH$), 85 (C_6H_{13}); no intense peak at *m/z* 127 (see that for a more polar isomer).

A more polar bromohydrin **6b** was obtained from the fractions eluted with ethyl acetate–*n*-hexane (20:80). Recrystallization of **6b** from 99% ethanol–dichloromethane gave fine needles (77 mg; 21.6% yield), mp 199~210°C; $[\alpha]_D^{24}$ −5.36° (*c* 0.634, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 3470 (m), 1736 (s), 1701 (s), 1455 (m), 1365 (m), 1263 (s), 1256 (s), 1240 (s), 1210 (w), 1165 (w), 1145 (w), 1118 (w), 1070 (w), 1035 (m), 978 (w). PMR (60 MHz; $CDCl_3$) δ: 0.74 (s, 3H), 0.8~2.8 (m), 1.97 (s, 3H), 2.07 (s, 3H), 4.06 (m, 2H), 4.95 (m, 1H), 5.33 (m, 1H). MS (*m/z*): a pair of molecular ion peaks was not observed; 566 and 564 (M^+-AcOH), 544 (M^+-HBr), 526 ($M^+-HBr-H_2O$), 501, 484 ($M^+-HBr-AcOH$), 459 ($M^+-HBr-C_6H_{13}$), 400 ($M^+-AcOH-C_6H_{13}Br$), 387 ($M^+-AcOH-C_6H_{13}CHBr$), 127 ($C_6H_{13}CHBrCHOH-HBr$), 85 (C_6H_{13}); no intense peak at *m/z* 115 (see that for a less polar isomer).

(2R,3S,24S)-2,3-Diacetoxy-22-bromo-24-ethyl-5α-cholestane-6,23-dione **7**. Jones reagent (30 μl) was added to a stirred solution of the less polar bromohydrin **6a** (34 mg) in acetone (2 ml) on an ice bath. After stirring was continued for 30 min, an excess amount of methanol was added to the reaction mixture. The mixture was evaporated under reduced pressure. The obtained residue was partitioned with water and ether. The ethereal layer was successively washed with water, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a gummy residue (26 mg; 76.5% yield) of **7**, showing a single spot on a TLC plate. Crystallization of **7** from 99% ethanol–ether gave colorless crystals, mp 114~118°C. ν_{max}^{nujol} (cm^{-1}): 1740 (s), 1700 (s), 1660 (m), 1458 (s), 1368 (s), 1270 (s), 1262 (s), 1230 (s), 1165 (w), 1148 (w), 1099 (w), 1036 (s), 1018 (m), 990 (w), 945 (w), 898 (w). PMR (100 MHz; $CDCl_3$) δ: 0.71 (s, 3H), 0.77 (s, 3H), 0.8~2.5 (m), 2.00 (s, 3H), 2.09 (s, 3H), 2.63 (m, 2H), 4.59 (m, 1H), 4.96 (m, 1H), 5.40 (m, 1H). CD: $[\theta]_{344.5\,nm}$ −10760. MS (*m/z*): 624 and 622 (M^+), 564 and 562 (M^+-AcOH), 542 (M^+-HBr), 457 ($M^+-HBr-C_6H_{13}$), 417 ($M^+-C_6H_{13}COCHBr$), 357 ($M^+-AcOH-C_6H_{13}COCHBr$), 297 ($M^+-2AcOH-C_6H_{13}COCHBr$), 113 ($C_6H_{13}CO^+$), 85 ($C_6H_{13}^+$).

(2R,3S,24S)-2,3,23-Triacetoxy-22-bromo-24-ethyl-5α-cholestan-6-one **8a**. A solution of the less polar bromohydrin **6a** (53 mg), acetic anhydride (2 ml) and 4-(*N,N*-dimethylamino)pyridine (5 mg) in dry pyridine (2 ml) was stirred for 42 hr at room temperature, poured into dilute hydrochloric acid and extracted three times with ether. The ethereal extracts were combined, successively washed twice with dilute hydrochloric acid, once with water, five times with aqueous sodium hydrogen carbonate and once with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The crystalline residue obtained was recrystallized from 99% ethanol–dichloromethane to give colorless fine needles (43 mg) of **8a**, mp 209~212°C; $[\alpha]_D^{24}$ −7.24° (*c* 0.649, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 1744 (s), 1700 (m), 1658 (m), 1460 (s), 1375 (s), 1365 (s), 1305 (w), 1256 (s), 1230 (s), 1172 (w), 1150 (w), 1115 (w), 1103 (w), 1077 (w), 1038 (m), 1018 (m), 960 (w), 900 (w), 859 (w). PMR (100 MHz; $CDCl_3$) δ: 0.68 (s, 3H), 0.7~2.7 (m), 2.00 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 4.28 (d, $J=11$ Hz, 1H), 4.95 (m, 1H), 5.38 (m, 1H). MS (*m/z*): a pair of molecular ion peaks was not observed; 608 and 606 (M^+-AcOH), 566 and 564 ($M^+-AcOH-CH_2=CO$), 533 and 531 ($M^+-2AcOH-Me$), 526 ($M^+-AcOH-HBr$).

(2R,3S,24S)-2,3,22-Triacetoxy-23-bromo-24-ethyl-5α-cholestan-6-one **8b**. A crystalline **8b** (80 mg) was obtained from the more polar bromohydrin **6b** (77 mg) in 97.6% yield in the same manner as **8a**. Recrystallization of **8b** from 99% ethanol–ether–dichloromethane gave colorless needles, mp 191~192°C; $[\alpha]_D^{24}$ −5.59° (*c* 0.608, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 1736 (s), 1709 (s), 1458 (m), 1372 (m), 1363 (s), 1300 (w), 1255 (s), 1245 (s), 1240 (s), 1230 (s),

1225 (s), 1215 (m), 1180 (w), 1150 (w), 1120 (w), 1100 (w), 1090 (w), 1072 (w), 1039 (s), 1017 (m), 989 (w), 968 (w), 945 (w), 940 (w), 930 (w), 898 (w), 880 (w), 730 (w). PMR (100 MHz; $CDCl_3$) δ: 0.71 (s, 3H), 0.8~2.8 (m), 1.98 (s, 3H), 2.07 (s, 6H), 4.23 (d, J=11 Hz, 1H), 4.93 (m, 1H), 5.37 (dd, J=4 and 7 Hz, 1H). MS (*m/z*): 668 and 666 (M^+), 608 and 606 (M^+ – AcOH), 526 (M^+ – AcOH–HBr), 483 (M^+ – AcOH–MeCO–HBr).

(2R,3S,22R,23R,24S)-24-Ethyl-2,3,22,23-tetrahydroxy-5α-cholestan-6-one **4b**. The diacetoxyenone **3** (264 mg; 0.5 mmol) was added to a stirred suspension of *N*-bromosuccinimide (134 mg; 0.75 mmol) and silver acetate (125 mg; 0.75 mmol) in glacial acetic acid. After stirring for 43 hr at room temperature, a mixture of glacial acetic acid (3 ml) and water (2 ml) was added to the reaction mixture and the mixture was then stirred for 22 hr at 90~100°C. After cooling, the reaction mixture was filtered and the solid residue was washed with ethyl acetate. Five volumes of water were added to the combined filtrate and washings and then partitioned. The organic layer was washed five times with aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give an orange-colored gum (287 mg). This was hydrolyzed with potassium hydroxide in aqueous methanol for 3 hr at reflux temperature. The mixture was poured into water, extracted twice with ethyl acetate, washed with brine, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a gummy residue. This was crystallized from 99% ethanol to give colorless needles (10 mg), mp 227~230°C. ν_{max}^{nujol} (cm^{-1}): 3500 (s), 3400 (s), 1695 (s), 1660 (sh), 1455 (s), 1400 (w), 1377 (s), 1343 (w), 1300 (w), 1219 (w), 1130 (m), 1080 (s), 1048 (s), 1040 (s), 1030 (sh), 1010 (m), 982 (m), 970 (w), 940 (w), 930 (w), 875 (w). PMR (100 MHz; $CDCl_3$–CD_3OD, 9:1) δ: 0.80 (s, 3H), 0.85~2.2 (m), 1.92 (s, 4H), 3.0~4.0 (m, 4H). CMR (25 MHz; $CDCl_3$–CD_3OD, 9:1) δ: 213.9, 74.5, 72.7, 68.4, 68.2, 56.9, 54.0 52.8, 51.1~46.9 (overlapped with signals of CD_3OD), 43.1, 42.9, 40.1, 39.7, 38.2, 37.3, 29.3, 27.8, 26.6, 24.1, 21.5, 21.3, 19.7, 19.2, 13.8, 13.7, 12.1. An unsatisfactory mass spectrum was obtained.

The mother liquor was concentrated to give a syrupy residue (88 mg) which was transformed into an acetonide form with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in dry dichloromethane. The material obtained was crystallized from 99% ethanol to give colorless needles of **2a**, mp 183~184°C.

(2R,3S,22R,23R,24S)-24-Ethyl-2,3,22,23-bis(isopropylidenedioxy)-5α-cholestan-6-one **2b**. A solution of the tetraol ketone **4b** (8 mg), 2,2-dimethoxypropane (2 ml) and *p*-toluenesulfonic acid (a catalytic amount) in dry dichloromethane (2 ml) was stirred for 1 hr at room temperature. The solution was washed with aqeuous sodium hydrogen carbonate, dried over anhydrous potassium carbonate and evaporated under reduced pressure to give a residue (6 mg). This was crystallized from 99% hot ethanol to give colorless needles, mp 140~141°C. PMR (400 MHz; $CDCl_3$) δ: 0.66 (s, 3H), 0.68 (s, 3H), 0.89 (d, J=7.1 Hz, 3H), 0.91 (d, J=6.8 Hz, 3H), 0.95 (t, J=7.6 Hz, 3H), 0.98 (d, J=6.6 Hz, 3H), 1.34 (s, 3H), 1.35 (s, 6H), 1.51 (s, 3H), 1.01~2.18 (m), 2.32 (dd, J=4.3 and 12.9 Hz, 1H), 2.55 (dd, J=4.0 and 12.8 Hz, 1H), 3.77 (dd, J=0.4 and 8.8 Hz, 1H), 3.81 (dd, J=2.8 and 8.8 Hz, 1H), 4.10 (ddd, J=4.9, 6.6 and 9.5 Hz, 1H), 4.28 (dt, J=1.7 and 4.4 Hz, 1H). MS (*m/z*): identical with that of **2a**; namely, 558 (M^+), 557 (M^+ – H), 544, 543 (M^+ – Me), 473 (M^+ – C_6H_{13}), 429 (M^+ – Me–C_6H_{13}CHO), 370. The LC analysis revealed less polarity of **2b** than **2a**.

(2R,3S,22S,23S,24S)-24-Ethyl-2,3,22,23-bis(isopropylidenedioxy)-5α-cholestan-6-one **2a**.

a) Pure form: a mixture of (2*R*,3*S*,22*E*,24*S*)-24-ethyl-2,3-dihydroxy-5α-cholest-22-en-6-one (mp 235~238°C)[5] (111 mg; 0.25 mmol), osmium tetroxide (63.5 mg; 0.25 mmol) and dry pyridine (1.0 ml)[32] was stirred for 24 hr in darkness under an argon atmosphere at room temperature. A solution of sodium hydrogen sulfite (120 mg) in a mixture of water (1.9 ml) and pyridine (1.3 ml) was added to the stirred reaction mixture. After stirring for 30 min, the mixture was extracted three times with chloroform. The combined extracts were successively washed twice with water, three times with dilute hydrochloric acid, once with water and once with aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate and evaporated under reduced pressure. A greenish residue was obtained. The residue was manipulated in the same manner as described in the preparation of **2b**. Chromatographic purification of the crude product (198 mg) gave crystalline **2a**. Recrystallization of **2a** from 99% hot ethanol gave colorless needles, mp 185~186°C; $[\alpha]_D^{22.5}$ +11.6° (*c* 0.110, $CHCl_3$). ν_{max}^{nujol} (cm^{-1}): 1715 (s), 1460 (s), 1375 (s), 1365 (s), 1330 (w), 1295 (w), 1265 (w), 1240 (s), 1210 (s), 1190 (w), 1170 (m), 1150 (w), 1120 (w), 1090 (w), 1080 (w), 1050 (s), 1015 (m), 980 (w), 960 (w), 930 (w), 910 (w), 880 (w), 835 (w), 820 (w), 785 (w). PMR (400 MHz; $CDCl_3$) δ: 0.67 (s, 3H), 0.68 (s, 3H), 0.95 (d, J=6.6 Hz, 3H), 0.96 (d, J=6.8 Hz, 3H), 0.97 (t, J=7.3 Hz, 3H), 1.03 (d, J=6.8 Hz, 3H), 1.34 (s, 3H), 1.37 (s, 6H), 1.50 (s, 3H), 1.09~2.18 (m), 2.32 (dd, J=4.3 and 13.1 Hz, 1H), 2.53 (dd, J=4.1 and 12.6 Hz, 1H), 3.90 (dd, J=3.2 and 8.6 Hz, 1H), 3.99 (dd, J=2.2 and 8.6 Hz, 1H), 4.10 (ddd, J=4.9, 6.6 and 9.5 Hz, 1H), 4.28 (dt, J=1.7 and 4.4 Hz, 1H). The mass spectrum of **2a** was identical with that of **2b**. *Anal.* Found; C: 74.61, H: 10.38. Calcd.; C: 75.22, H: 10.46.

b) Crude form: (22*E*,24*S*)-24-ethyl-5α-cholesta-2,22-dien-6-one[7] (14.3 g; 0.035 mol) was manipulated with osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide in aqueous acetone for 8 hr, according to Van Rheenen *et al.*[32] The resulting crystals of (2*R*,3*S*,22*E*,24*S*)-24-ethyl-2,3-dihydroxy-5α-cholest-22-en-6-one[5] (7.3 g) were filtered off. After additional manip-

ulation of the filtrate, the second crop of the enone (2.2 g) separated from the acetone solution. A mother liquor was concentrated to give dark-colored syrup (9.0 g). This was transformed into an acetonide form in the same manner as described in the preparation of **2b**. After chromatographic purification, **2a** (3.1 g; 15.9% overall yield from the starting dienone) was obtained along with a small amount of (2*R*,3*S*,22*E*,24*S*)-24-ethyl-2,3-isopropylidenedioxy-5α-cholest-22-en-6-one,[5,11] mp 158~159°C (recrystallization from 99% hot ethanol); $[\alpha]_D^{24}$ +21.1° (*c* 0.645, $CHCl_3$). The **2a** obtained was recrystallized from 99% hot ethanol to give crystals, mp 135~146°C. The LC analysis revealed the crystals to be an admixture containing two other unknown compounds.

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