Synthesis of Brassinolide Analogs and Their Plant Growth-promoting Activity[†]

Katsuhide OKADA and Kenji MORI

Department of Agricultural Chemistry, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

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Brassinolide analogs such as (22*S*,23*S*)-28-norbrassinolide **8b**, a lactam (6-aza-7-keto analog) **12b**, a thiolactam (6-aza-7-thiono analog) **14b** and an isomer (6-oxa-7-keto isomer) **15b** of (22*S*,23*S*)-homobrassinolide were synthesized. Only two of them (**8b** and **15b**) were bioactive.

The discovery of brassinolide **1** as a potent plant growth-promotor has aroused much interest among synthetic chemists.¹⁾

Since then a number of syntheses of brassino-steroids have been reported,^{2~9)} including three different syntheses of brassinolide itself.^{4,5,8)} One of the interesting aspects of brassinolide research is the clarification of the structure-activity relationship. Indeed the pioneering works of Thompson *et al.*²⁾ and ours³⁾ have indicated that some analogs are highly bioactive. Later Ikekawa *et al.*,⁶⁾ Wada and Marumo⁷⁾ and ourselves⁸⁾ have reported other examples of bioactive brassino-steroids. Here we describe our work along this line leading to the syntheses of (22*S*,23*S*)-28-

norbrassinolide and other analogs containing hetero-atom(s) such as nitrogen and sulfur.

Since the higher homolog of brassinolide, with an ethyl group at C-24 (homobrassinolide^{3,7~9)}) instead of the methyl group in **1**, was bioactive,^{3,10)} we became interested in removing the methyl group at C-24 so as to know the contribution of an alkyl substituent at C-24 to biological activity. Our first target was therefore 28-norbrassinolide **8b** (Scheme 1). The synthesis started from stigmasterol which was converted to a known ketone **2a**.^{3,8)} The corresponding olefinic acetal **2b** was cleaved by ozonolysis to give an aldehyde **3**. Reconstruction of the steroid side chain was achieved by the method developed by

SCHEME 1. Synthesis of (22*S*,23*S*)-28-Norbrassinolide.

[†] Brassinolide and Its Analogs. Part III. For Part II, see ref. 9.

Kocienski *et al.*^{11,12)}

A carbanion derived from 3-methylbutylphenylsulfone was condensed with **3** giving a β -acetoxy sulfone **4** after acetylation. Reduction of **4** with sodium-amalgam yielded **5b**.

Deprotection of **5b** with acetic acid gave an olefinic ketone, **5a**, whose $^1\text{H-NMR}$ spectrum confirmed the (*E*)-geometry of the double bond on the basis of a large $J_{\text{H}_{22},\text{H}_{23}}$ value (15.1 Hz) as was observed in the case of **2a** ($J_{\text{H}_{22},\text{H}_{23}} = 15.1$ Hz). Hereafter the synthesis followed the route previously employed for the synthesis of homobrassinolide.^{3,8)}

The ketone **5a** thus yielded a dienone **6**, whose oxidation with osmium tetroxide and *N*-methylmorpholine *N*-oxide¹³⁾ afforded a tetrahydroxy ketone **7b** as a single isomer which was proved by the HPLC analysis of its bis-acetonide.^{cf.9)} The corresponding acetate **7a** was submitted to the Baeyer-Villiger oxidation with trifluoroperacetic acid to give 28-norbrassinolide tetraacetate **8a** and its regioisomer **9** in a ratio of 10:1. The structures were assigned on the basis of their $^1\text{H-NMR}$ spectra which were similar to those reported for homobrassinolide tetraacetate^{3,8)} and its regioisomer **15a**.⁷⁾ Saponification of **8a** was followed by acidification to yield **8b**, mp 129.5~133.5°C, $[\alpha]_{\text{D}}^{23} + 29.6^\circ$ (methanol), which was different from (22*R*,23*R*)-28-norbrassinolide, mp 256~259°C, $[\alpha]_{\text{D}}^{14} + 32^\circ$ (methanol).⁶⁾ Professor N. Ikekawa kindly informed us of his unpublished data on (22*S*,23*S*)-28-norbrassinolide, mp 130~

135°C, $[\alpha]_{\text{D}}^{16} + 28.3^\circ$ (methanol). Comparison of these data proved the common identity of his material and ours.

This implies that the hydroxylation step was stereoselective, yielding a (22*S*,23*S*)-diol system as in another case.⁹⁾

We then turned our attention to the synthesis of an aza-analog of brassinolide by the Beckmann rearrangement of an oxime **11** which had been prepared from the known tetraacetoxy ketone **10a** in the conventional manner. Treatment of **11** with tosyl chloride in pyridine yielded only one lactam **12a**. An isomeric lactam **13** could not be detected. The same lactam **12a** was obtained directly from **10a** by the Schmidt reaction employing sodium azide and methanesulfonic acid. Saponification of **12a** yielded as aza-analog **12b** of homobrassinolide. The assigned structures **12a** and **12b** were supported by the $^1\text{H-NMR}$ measurements of the lactams at 400 MHz. Irradiation of the N-H of **12b** at δ 7.75 decoupled the H-C(5)-N-H spin-spin interaction and the signal due to C(5)-H was observed as a quartet ($J_1 = 6$, $J_2 = 14$ Hz). Without irradiation the signal due to C(5)-H was seemingly a quintet. Similar NMR data were also obtained for **12a**. The regio-selective formation of the 6-aza-B-homo-steroid **12a** by the Beckmann rearrangement is in accord with Knof's result on the Beckmann rearrangement of 3 β -acetoxy-6-cholestanone oxime.¹⁴⁾

The recent advent of a Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide] as an efficient tool

SCHEME 2. Syntheses of Brassinolide Analogs Substituted with Hetero-atoms.

for the thiation of different classes of carbonyl compounds including lactams^{15,16}) made us attempt the conversion of the lactam **12a** to a thiolactam **14a**. After the successful conversion in a 97% yield, the resulting **14a** was hydrolysed to a thiolactam analog **14b** of homobrassinolide.

The plant growth-promoting activity of the above mentioned brassinolide analogs was examined by Maeda's lamina-inclination test using rice-seedlings.¹⁷) In addition to the compounds **7b**, **8b**, **10b**, **12b** and **14b**, a 6-oxa-isomer **15b** of (22*S*,23*S*)-homobrassinolide was also tested. This compound was obtained as a by-product in the course of our large-scale synthesis of (22*S*,23*S*)-homobrassinolide for the field test. The Baeyer–Villiger oxidation of **10a** yielded 6.6% of **15a**,¹⁰) which yielded **15b** upon hydrolysis and acidification.

The result of the bioassay is shown in Table I. For the purpose of comparison, brassinolide itself was also assayed, which proved to be the most active one. (22*S*,23*S*)-28-Norbrassinolide **8b** and the tetrahydroxy ketone **10b** were active but less effective than brassinolide. The 6-oxa analog **15b** and the tetrahydroxy-cholestanone **7b** were of marginal activity.

The lactam **12b** and the thiolactam **14b** were entirely inactive. This indicates the importance of i) the ring B-lactone or ketone systems, ii) the alkyl substituent at C-24, and iii) the correct absolute configuration of the two hydroxyl groups at C-22 and C-23 for high biological activity.

EXPERIMENTAL

All melting points were uncorrected. IR spectra were determined on a Jasco A-102 spectrometer. ¹H-NMR spectra were recorded on a Hitachi R-24A spectrometer at 60 MHz, on a JEOL JNM-MH-100 spectrometer at 100 MHz and on a JEOL FX-400 spectrometer at 400 MHz with TMS as an internal standard. ¹³C-NMR spectra were recorded on a JEOL FX-100 spectrometer at 25 MHz. Optical rotations were measured on a Jasco DIP-140 polarimeter.

(22*E*,24*S*)-3*α*,5-Cyclo-24-ethyl-5*α*-cholesta-22-en-6-one ethyleneneacetal **2b**. To a solution of cyclopropylketone (**2a**, 24.5 g) and 2-methoxy-1,3-dioxolane (60 g) in dry

TABLE I. EFFECT OF THE BRASSINOLIDE ANALOGS ON RICE LAMINA INCLINATION*

Compound	Concentration (ppm)	Angle in degrees between laminae and sheaths (average)
Control		32.6
1 Brassinolide	0.001	92.0
	0.01	106.2
	0.1	140.2
7b	0.001	28.6
	0.01	53.3
	0.1	32.2
8b	0.001	28.4
	0.01	31.0
	0.1	89.3
10b	0.001	42.8
	0.01	43.8
	0.1	75.5
12b	0.001	35.1
	0.01	33.4
	0.1	32.0
14b	0.001	38.2
	0.01	32.9
	0.1	32.2
15b	0.001	38.3
	0.01	39.4
	0.1	56.3

* For experimental procedure see ref. 17.

CH₂Cl₂ (300 ml), a drop of conc. H₂SO₄ was added at room temperature and the solution was left overnight. The mixture was poured into a 20% K₂CO₃ solution (300 ml), extracted by ether and the extract was dried over K₂CO₃. Removal of the solvent gave 27 g of crude **2b**, which was chromatographed on alumina and eluted with *n*-hexane to give 26 g of **2b** (95%), *n*_D^{24.5} 1.5075; [α]_D²⁴ +31.9 (*c*=1.40, CHCl₃); MS (*m/z*): 454 (M⁺); IR $\nu_{\max}$ cm⁻¹ (film): 1460 (m), 1380 (m), 1170 (m), 1100 (m), 1080 (m), 980 (m); NMR δ (60 MHz, CDCl₃): 0.7~1.1 (CH₃ × 6), 1.1~2.2 (26H), 3.7~4.0 (4H), 4.9~5.1 (2H); Anal. Found: C, 81.90; H, 11.20, Calcd. for C₃₁H₅₀O₂: C, 81.88%; H, 11.08%.

(20*S*)-3*α*,5-Cyclo-20-formyl-5*α*-pregna-6-one ethyleneacetal **3**. Ozone was introduced to a solution of cyclopropylacetal (**2b**, 21.8 g) and NaHCO₃ (8 g) in dichloromethane (800 ml) and methanol (1200 ml) at -55°C. Me₂S (100 g) was then added to the solution and the tem-

perature was raised gradually to room temperature. The solution was poured into ether, and the ether layer was washed twice with water, dried over MgSO_4 and evaporated. The residue was purified by chromatography (Mallinckrodt CC-7 *n*-hexane-ether=95:5) to give 16.6 g (93%) of **3**, $[\alpha]_D^{24} + 40.5$ ($c=3.28$, CHCl_3); MS (m/z): 372 (M^+); IR ν_{max} cm^{-1} (film): 2700 (m), 1730 (s), 1460 (m), 1380 (m), 1160 (m), 1080 (s), NMR δ (60 MHz, CDCl_3): 0.7~1.2 ($\text{CH}_3 \times 3$), 1.2~2.2 (22H), 3.7~4.0 (4H), 9.5 (1H, d, $J=4$ Hz).

(22*E*)-3 α ,5-Cyclo-5 α -cholesta-22-en-6-one ethylene-acetal **5b**. To a stirred solution of the 3-methylbutyl-phenyl sulfone (1.00 g) in THF (30 ml) under argon, 1.1 M-*n*-butyllithium in hexane (4.7 ml) was added. After 25 min the aldehyde (**3**, 1.60 g) in THF (25 ml) was added dropwise. Stirring was continued at the same temperature for 90 min and then acetic anhydride (880 mg) was added. After 5 hr the mixture was allowed to attain room temperature and stirring was continued overnight. Saturated aqueous ammonium chloride was then added, the mixture was extracted with ether, and the extract was washed with water, dried, and evaporated to give the oily acetoxysulfone (**4**). It was used immediately in the next step. A solution of the acetoxysulfone in methanol (20 ml) and ethyl acetate (15 ml) was stirred at -20°C with 6% sodium amalgam (10.6 g) for 10 hr. The mixture was isolated with ether and chromatographed on a column of silica gel (20 g) and *n*-hexane to give 980 mg (53%) of **5b**, $n_D^{23.5} 1.5108$; $[\alpha]_D^{23.5} + 34.2$ ($c=1.25$, CHCl_3); MS (m/z): 426 (M^+); IR ν_{max} cm^{-1} (film): 1460 (m), 1380 (m), 1160 (m), 1100 (m), 1070 (m), NMR δ (60 MHz, CDCl_3): 0.7~1.2 ($\text{CH}_3 \times 5$), 1.2~2.2 (25H), 3.7~4.0 (4H), 5.1~5.3 (2H).

(22*E*)-3 α ,5-Cyclo-5 α -cholesta-22-en-6-one **5a**. A mixture of acetal (**5b**, 8.8 g), acetic acid (30 ml) and water (10 ml) in THF (80 ml) was stirred overnight at room temperature. The solution was poured into water, extracted with ether three times and the extract was washed with saturated aqueous NaHCO_3 solution three times, water twice, dried over Na_2SO_4 and evaporated to give 7.5 g of **5a** (96%), mp $87 \sim 87.5^\circ\text{C}$ (EtOH); $[\alpha]_D^{24} + 18.3$ ($c=0.41$, CHCl_3); MS (m/z): 382 (M^+); IR ν_{max} cm^{-1} (nujol): 1690 (s), 1300 (m), 1170 (w), 1150 (w), 970 (m); NMR δ (60 MHz, CDCl_3): 0.7~1.2 ($\text{CH}_3 \times 5$), 1.2~2.2 (25H), 5.1~5.3 (2H); NMR δ (400 MHz, CDCl_3): $J_{\text{H}_{22},\text{H}_{23}} = 15.1$ Hz, cf. $J_{\text{H}_{22},\text{H}_{23}} = 15.1$ Hz in 24*S*-ethyl compound (**2a**); Anal. Found: C, 84.91; H, 11.26, Calcd. for $\text{C}_{27}\text{H}_{42}\text{O}$: C, 84.75; H, 11.07%.

(22*E*)-5 α -Cholesta-2,22-dien-6-one **6**. A solution of ketone (**5a**, 7.2 g) and *p*-TsOH (400 mg) in sulfolane (40 ml) was heated at 160°C for 70 min under argon and then was poured into water. The mixture was extracted by benzene and ether (1:1) and the extract was washed with water, brine and dried over MgSO_4 . Removal of the solvent gave a residue which was chromatographed on silica gel and

benzene-*n*-hexane (1:1) to give 3.5 g (49%) of **6**, mp $72 \sim 72.5^\circ\text{C}$ (EtOH); $[\alpha]_D^{23.4} + 21.6$ ($c=1.00$, CHCl_3); MS (m/z): 382 (M^+); IR ν_{max} cm^{-1} (nujol): 3020 (m), 1707 (s), 1650 (w), 965 (m); NMR δ (60 MHz, CDCl_3): 0.7~1.2 ($\text{CH}_3 \times 5$), 1.2~2.5 (23H), 5.1~5.3 (2H), 5.5~5.65 (2H); Anal. Found: C, 85.06; H, 11.07, Calcd. for $\text{C}_{27}\text{H}_{42}\text{O}$: C, 84.75; H, 11.07%.

(2*R*,3*S*,22*S*,23*S*)-2,3,22,23-Tetrahydroxy-5 α -cholestan-6-one **7b**. To a stirred solution of **6** (2.3 g), *N*-methylmorpholine *N*-oxide (2 g) and water (12 ml) in THF (40 ml) was added OsO_4 (200 mg) in *t*-BuOH (8 ml) under argon. After 2 days *N*-methylmorpholine *N*-oxide (1 g) was added and then after a day 1 g of *N*-methylmorpholine *N*-oxide was added. The solution was stirred for total 4 days and aqueous $\text{Na}_2\text{S}_2\text{O}_4$ (2 g in 4 ml of water) was added. The mixture was filtered through Celite and the black residue was concentrated. Chloroform was added to the residue which was washed with dil. HCl and dried over K_2CO_3 . Removal of the solvent gave a residue which was chromatographed on silica gel and chloroform-MeOH (100:1) to give 1.3 g (48%) of **7b**, $[\alpha]_D^{24} + 0.60$ ($c=5.56$, CHCl_3 -MeOH = 1:1); MS (m/z): 450 (M^+); IR ν_{max} cm^{-1} (nujol): ~ 3350 (s), 1710 (s), 1080 (m), 1050 (s), 1020 (m). The purity of this compound was checked by conversion to bis-acetonide.

Bis-acetonide of tetraol (**7b**). A solution of **7b** (150 mg) and *p*-TsOH (30 mg) in dimethoxy propane (5 ml) was left overnight at room temperature and was poured into saturated NaHCO_3 solution. The solution was extracted with ether, and the extract was dried over K_2CO_3 .

The product, after removal of the solvent, was purified by preparative TLC (*n*-hexane-AcOEt = 100:15) to give 88 mg of bis-acetonide, $[\alpha]_D^{24} + 14.2$ ($c=0.84$, CHCl_3); IR ν_{max} cm^{-1} (film): 1710 (s), 1460 (m), 1380 (s), 1370 (s), 1240 (s), 1210 (s), 1170 (m), 1050 (s), 1020 (m); NMR δ (100 MHz, CDCl_3): 0.7~1.4 ($\text{CH}_3 \times 9$), 1.1~2.3 (23H), 3.8 (2H), 4.3 (2H); ^{13}C -NMR (CDCl_3): 210.9 (C=O), 107.84, 106.91 ($\text{C} \begin{smallmatrix} \diagup \text{O} \\ \diagdown \text{O} \end{smallmatrix}$), 82.78, 74.35, 72.28 (C $\times 2$), (C-O);

HPLC (Shimadzu LC-2, Partisil-5, $1/2'' \times 4.6 \times 250$ mm, RI 16×10^{-5} , 2 ml/min, *n*-hexane-AcOEt = 5:1): t_R 19 min.

(2*R*,3*S*,22*S*,23*S*)-2,3,22,23-Tetraacetoxy-5 α -cholestan-6-one **7a**. To a solution of tetraol (**7b**, 980 mg) in dry pyridine (10 ml) was added acetic anhydride (3 ml) and DMAP (0.3 g). The reaction mixture was stirred at room temperature for 12 hr, poured into ice-dil. HCl, and extracted with ether. The organic solvent was washed with water, saturated NaHCO_3 solution and brine, and dried over MgSO_4 . The solvent was evaporated to give a residue which was chromatographed on silica gel and *n*-hexane-AcOEt = 9:1~1:1 to give 940 mg (70%) of oily **7a**, $[\alpha]_D^{23} - 16.5$ ($c=1.5$, CHCl_3); MS (m/z): 618 (M^+); IR ν_{max} cm^{-1} (film): 1740 (s), 1710 (m), 1230 (s), 1040 (m), 1020

(s), 960 (s), 900 (s); NMR δ (60 MHz, CDCl_3): 0.7~1.1 ($\text{CH}_3 \times 5$), 1.1~2.5 (23H), 1.95 (3H, s), 2.0 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 4.8~4.9 (2H), 5.3~5.4 (2H); *Anal.* Found: C, 67.73; H, 8.80, Calcd. for $\text{C}_{35}\text{H}_{54}\text{O}_9$: C, 67.93; H, 8.80%.

(2*R*,3*S*,22*S*,23*S*)-2,3,22,23-Tetraacetoxy-B-homo-7-oxa-5 α -cholestan-6-one **8a**. Trifluoroacetic anhydride (5 ml) was added dropwise to an ice cooled solution of 90% H_2O_2 (0.8 ml) in dry CH_2Cl_2 (8 ml).

This solution of peracid was added dropwise to a mixture of tetraacetoxy ketone (**7a**, 900 mg) and Na_2HPO_4 (powder, 5 g) in dry CH_2Cl_2 (45 ml) on an ice-water bath under stirring. After 30 min the solution was refluxed for 1.5 hr and water was added to the solution after cooling. The aqueous layer was extracted with CH_2Cl_2 , the combined organic solvent was washed with saturated NaHCO_3 solution and brine and then dried over MgSO_4 . Removal of the solvent gave a product which was separated by chromatography (silica gel, *n*-hexane-AcOEt = 9:1~4:1) to give the recovery of **7a** (420 mg, 47%), **8a** (317 mg, 64%) and another product (40 mg).

Preparative TLC of this compound (*n*-hexane-AcOEt = 4:6) gave the isomer **9**, (27 mg, 5.5%). **8a**, $[\alpha]_D^{24} + 16.7$ ($c = 1.07$, CHCl_3); MS (m/z): 634 (M^+); IR $\nu_{\text{max}} \text{ cm}^{-1}$ (CHCl_3): 1740 (s), 1370 (m), 1190 (m), 1140 (m), 1050 (m), 1100 (m); NMR δ (60 MHz, CDCl_3): 0.7~1.1 ($\text{CH}_3 \times 5$), 1.1~2.1 (20H), 2.0 (3H, s), 2.05 (3H, s), 2.1 (6H, s), 3.0 (1H, m, $\text{CHC}=\text{O}$), 4.1 (2H, d, $J = 6$ Hz, CH_2O), 4.8~5.0 (2H, CHOAc), 5.2~5.4 (2H, CHOAc). **9**, $[\alpha]_D^{24} - 4.40$ ($c = 1.19$, CHCl_3); MS (m/z): 634 (M^+), 574 ($\text{M}^+ - 60$); IR $\nu_{\text{max}} \text{ cm}^{-1}$ (film): 1740 (s), 1370 (m), 1230 (s), 1040 (m), 1020 (m); NMR δ (100 MHz, CDCl_3): 0.7~1.1 ($\text{CH}_3 \times 5$), 1.1~2.7 (20H), 2.5 (2H, m, $\text{CH}_2\text{C}=\text{O}$), 4.5 (1H, d-d, $J = 10$ and 7 Hz, C(5)-H), 5.0 (2H, CHOAc), 5.5 (2H, CHOAc).

(2*R*,3*S*,22*S*,23*S*)-2,3,22,23-Tetrahydroxy-B-homo-7-oxa-5 α -cholestan-6-one ((2*S*,23*S*)-28-Norbrassinolide) **8b**. A solution of lactone (**8a**, 253 mg) and 45% NaOH solution (1 ml) in methanol (6 ml) was refluxed for 1 hr and THF (6 ml) was added to the solution. The mixture was acidified with 6 N-HCl, stirred overnight at 0°C and at room temperature for 2 hr. The solution was poured into saturated NaHCO_3 solution and extracted by CHCl_3 . The organic solution was washed with water and dried over Na_2SO_4 . Removal of the solvent gave 185 mg (88%) of **8b**, which was recrystallized from 99% EtOH to give needles, mp 129.5~133.5°C (130~135°C, data from Professor Ikekawa); $[\alpha]_D^{24} + 23.5$ ($c = 1.17$, CHCl_3), $[\alpha]_D^{23} + 29.6$ ($c = 0.46$, MeOH) ($[\alpha]_D^{16} + 28.3$ (MeOH), data from Professor Ikekawa); IR $\nu_{\text{max}} \text{ cm}^{-1}$ (nujol): ~3400 (s), 1695 (s), 1405 (m), 1320 (m), 1260 (m), 1205 (m), 1120 (m), 1070 (s), 1020 (m), 995 (m); NMR δ (100 MHz, CDCl_3): 0.7~1.1 ($\text{CH}_3 \times 5$), 1.1~2.1 (20H), 3.1 (1H, m, $\text{CHC}=\text{O}$), 3.4 (2H), 3.7 (2H), 4.1 (2H); ^{13}C -NMR ($\text{C}_5\text{D}_5\text{N}$): 176.6 ($c = 0$), 75.6, 70.3, 68.7, 68.3, 68.1 (C-O); *Anal.* Found: C, 67.05;

H, 9.71, Calcd. for $\text{C}_{27}\text{H}_{46}\text{O}_6 + \text{H}_2\text{O}$: C, 66.91; H, 9.98%.

(2*R*,3*S*,22*S*,23*S*,24*S*)-2,3,22,23-Tetraacetoxy-24-ethyl-6-oximino-5 α -cholestan-11. A solution of the ketone (**10a**, 400 mg), pyridine (2 ml) and $\text{NH}_2\text{OH} \cdot \text{HCl}$ (500 mg) in 99% EtOH (2 ml) was refluxed for 2 hr and poured into water after cooling. The mixture was extracted by ethyl acetate and the extract was washed with water and dried over Na_2SO_4 . Removal of the solvent gave a product which was purified by chromatography on silica gel and *n*-hexane-AcOEt (4:1) to give 398 mg (97%) of **11**, $[\alpha]_D^{23.5} - 27.5$ ($c = 1.04$, CHCl_3); IR $\nu_{\text{max}} \text{ cm}^{-1}$ (CHCl_3): 3400 (m), 1740 (s), 1650 (w), 1460 (w), 1430 (w), 1370 (s), 1230 (s), 1040 (s), 1020 (s), 950 (w), 910 (w); NMR δ (60 MHz, CDCl_3): 0.7~1.1 ($\text{CH}_3 \times 6$), 1.1~3.2 (24H), 2.0 (3H, s), 2.05 (3H, s), 2.1 (6H), 5.0 (2H), 5.3 (2H), 9.0 (1H, NH).

(2*R*,3*S*,22*S*,23*S*,24*S*)-2,3,22,23-Tetraacetoxy-24-ethyl-B-homo-6-aza-5 α -cholestan-7-one **12a**. A solution of the oxime (**11**, 390 mg) and *p*-TsCl (400 mg) in pyridine (2 ml) was heated at 100°C for 1 hr and was poured into a 6 N-HCl solution after cooling. The mixture was extracted by ethyl acetate and the extract was washed with water and dried over Na_2SO_4 . The product, after removal of the solvent, was purified by chromatography (silica gel-AcOEt) to give 290 mg (74%) of **12a**, $[\alpha]_D^{24} + 13.9$ ($c = 0.90$, CHCl_3); MS (m/z): 661 (M^+); IR $\nu_{\text{max}} \text{ cm}^{-1}$ (CHCl_3): 3200 (w), 1740 (s), 1665 (s), 1460 (m), 1370 (s), 1230 (s), 1040 (m), 1020 (m); NMR δ (400 MHz, CDCl_3): 0.69 (3H, s), 0.85 (d, 3H), 0.9~0.95 ($\text{CH}_3 \times 4$), 1.1~1.95 (21H), 2.0 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 2.12 (3H, s), 2.3 (2H), 3.6 (quintet, 1H, C(5)-H), 4.85 (1H, OCH), 5.0 (1H, OCH), 5.2 (1H, OCH), 5.3 (1H, OCH), 5.97 (1H, br, NH). Decoupling of the NH proton at 5.97 causes the quintet at 3.6 (C(5)-H) to d-d ($J = 14$ and 6 Hz).

Schmidt reaction of 10a. To a stirred solution of the ketone (**10a**, 30 mg) and methanesulfonic acid (0.03 ml) in acetic acid (1 ml) was added a sodium azide (5 mg) at room temperature. After 2 hr the solution was poured into saturated NaHCO_3 solution and extracted with ethyl acetate. The extract was washed with water and dried over MgSO_4 . Removal of the solvent gave 25 mg of **12a**, whose IR spectra was identical with that of the product obtained by the Beckmann rearrangement.

(2*R*,3*S*,22*S*,23*S*,24*S*)-24-Ethyl-2,3,22,23-tetrahydroxy-B-homo-6-aza-5 α -cholestan-7-one **12b**. A solution of the tetraacetate (**12a**, 70 mg) and 40% NaOH aqueous solution (1.5 ml) in methanol (3 ml) was refluxed for 40 min and poured into water. The mixture was extracted with chloroform, and the extract was washed twice with water and dried over Na_2SO_4 . Removal of the solvent gave a solid which was recrystallized from CHCl_3 and MeOH to give 50 mg (quantitative) of **12b**, mp 150~155°C and 261~264°C; $[\alpha]_D^{23.5} + 32.8$ ($c = 0.72$, $\text{C}_5\text{H}_5\text{N}$); IR $\nu_{\text{max}} \text{ cm}^{-1}$ (nujol): 3600 3100 (s), 1655 (s), 1650 (s), 1300 (w),

1240 (w), 1070 (m), 1050 (m), 1020 (m); NMR δ (400 MHz, C_5D_5N): 0.65 (CH_3), 1.0 (CH_3), 1.05 (d, CH_3), 1.1~1.15 ($CH_3 \times 3$), 1.2~2.6 (23H), 3.92 (1H, OCH), 3.97 (1H, OCH), 4.05 (1H, OCH), 4.19 (quintet, 1H, C(5)-H), 4.45 (1H, OCH), 7.75 (d, $J=6$ Hz, NH). Decoupling the NH proton at 7.75 causes the quintet at 4.19 (C(5)-H) to d-d ($J=14$ and 6 Hz). ^{13}C -NMR (C_5D_5N): 176.84 (C=O), 72.25, 70.32, 69.12, 68.54 (C-O); *Anal.* Found: C, 68.61; H, 10.22; N, 2.50. Calcd. for $C_{29}H_{51}O_5N + CH_3OH$: C, 68.53; H, 10.54; N, 2.66%.

(2*R*,3*S*,22*S*,23*S*,24*S*)-2,3,22,23-Tetraacetoxy-24-ethyl-7-thioxo-B-homo-6-aza-5 α -cholestane **14a**. A solution of lactam (**12a**, 110 mg) and [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide] (Lawesson's Reagent^{15,16}) (37 mg) in dry benzene (2 ml) was refluxed for 2 hr under argon. The benzene was then evaporated and the residue purified by chromatography (silica gel and benzene-AcOEt=3:1) to give 109 mg (97%) of **14a**, $[\alpha]_D^{22.5} + 56.03$ ($c=2.65$, $CHCl_3$); MS (m/z): 678 (M^+); IR ν_{max} cm^{-1} (film): 3300 (m), 3200 (m), 1740 (s), 1540 (s), 1440 (s), 1370 (s), 1230 (s), 1120 (s), 1040 (s), 1020 (s), 950 (m); NMR δ (60 MHz, $CDCl_3$): 0.7~1.1 ($CH_3 \times 6$), 1.1~2.0 (23H), 1.98 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 2.10 (3H, s), 3.75 (1H, C(5)-H), 4.95 (2H), 5.25 (2H), 8.0 (1H, d, NH).

(2*R*,3*S*,22*S*,23*S*,24*S*)-24-Ethyl-2,3,22,23-tetrahydroxy-7-thioxo-B-homo-6-aza-5 α -cholestane **14b**. A solution of the tetraacetate (**14a**, 100 mg) and 45% NaOH aqueous solution (0.8 ml) in methanol (3 ml) was refluxed for 5 min and poured into water. The mixture was extracted with chloroform and the extract was washed with water and dried over $MgSO_4$. After removal of the solvent, the product was purified by chromatography (silica gel and $CHCl_3$ -MeOH=8:1) to give 52 mg (69%) of **14b**, $[\alpha]_D^{23.5} + 127$ ($c=0.69$, C_5H_5N); IR ν_{max} cm^{-1} ($CHCl_3$): ~3200 (s), 1530 (s), 1460 (w), 1440 (w), 1380 (w), 1320 (w), 1120 (m), 1050 (m), 1020 (m); NMR δ (400 MHz, C_5D_5N): 0.7 (CH_3), 1.02 (CH_3), 1.03~1.15 ($CH_3 \times 4$), 1.15~2.55 (21H), 2.85 (1H, t, $J=12$ Hz, C(7)-H), 3.3 (1H, d, $J=14$ Hz, C(7)- β H), 3.95 (1H, HCO), 3.98 (1H, HCO), 4.05 (1H, HCO), 4.40 (1H, m, C(5)-H. Addition of D_2O causes multiplet to d-d.), 4.48 (1H, HCO), 5.05 (1H, OH), 5.58 (1H, OH), 6.10 (1H, OH), 6.30 (1H, OH), 10.30 (1H, d, $J=8$ Hz, NH); ^{13}C -NMR (C_5D_5N): 207.51 (C=S), 72.34, 70.35, 68.92, 68.30 (C-O).

(2*R*,3*S*,22*S*,23*S*,24*S*)-2,3,22,23-Tetraacetoxy-24-ethyl-B-homo-6-oxa-5 α -cholestan-7-one **15a**. The tetraacetate ketone (**10a**) was oxidized as described in ref.³) and then the isomeric lactone (**15a**) was obtained as a by-product in a 6.6% yield. (Separation by chromatography (silica gel and *n*-hexane-AcOEt 4:1~3:1)), $[\alpha]_D^{24} + 2.54$ ($c=1.11$, $CHCl_3$); MS (m/z): 662 (M^+); IR ν_{max} cm^{-1} ($CHCl_3$): 1740 (s), 1460 (w), 1370 (m), 1050 (m), 1020 (m); NMR (100 MHz, $CDCl_3$): 0.7~1.1 ($CH_3 \times 6$), 1.1~2.5 (23H), 2.0 (3H, s), 2.05 (3H, s), 2.0 (3H, s), 2.11 (3H, s), 4.5 (1H, d-d, $J=11$

and 7 Hz), 4.9 (1H), 5.0 (1H), 5.3 (1H), 5.4 (1H).

(2*R*,3*S*,22*S*,23*S*,24*S*)-24-Ethyl-2,3,22,23-tetrahydroxy-B-homo-6-oxa-5 α -cholestan-7-one **15b**. The acetate (**15a**) was saponified as in the case of **8a** to give **15b** in a 70% yield, mp 191~192°C (CH_3OH), $[\alpha]_D^{23} + 34.07$ ($c=0.73$, $CHCl_3$); IR ν_{max} cm^{-1} (nujol): ~3300 (s), 1720 (s), 1710 (s), 1330 (m), 1200 (m), 1170 (m), 1070 (m), 1060 (m), 1040 (m), 980 (m); NMR δ (100 MHz, $CDCl_3$): 0.7~1.1 ($CH_3 \times 6$), 1.1~2.6 (23H), 3.7 (m), 4.1 (m); ^{13}C -NMR ($CDCl_3$): 175.68 (C=O), 72.34, 70.73 (C $\times$ 2), 70.23, 68.83 (C-O); *Anal.* Found: C, 68.11; H, 10.19. Calcd. for $C_{29}H_{50}O_6 + H_2O$: C, 67.93; H, 10.22%.

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