Preparation of New Derivatives from α-Dihydro Grayanotoxin-II

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Rhodojaponin-III and 10,14-epoxy-10-deoxy grayanotoxin-III were derived from grayanotoxin-I and -III, respectively. Preparation of 13 new derivatives from α-dihydro grayanotoxin-II was discussed. Some of them showed higher physiological activity than that of natural grayanotoxins.

Many grayanotoxins have been isolated from the leaves of the family of *Ericaceae*. Grayanotoxin-I (GTX-I),[1] the main toxic substance in *Leucothoe grayana* Max., has been found to depolarize excitable membranes owing to a specific increase in resting membrane permeability to sodium ions.[2] α-Dihydro-GTX-II (α-H_2GTX-II)[3] is a derivative from GTX-II, which shows the same effect as GTX-I on squid axon membranes,[4] and thus it is used as a tool for studing membrane depolarization. Recently, we have reported the structure-activity relationship between 34 GTX derivatives and their critical concentration for generation of action potential on frog skeletal muscle.[5] In this bioassay, 12 derivatives showed the remarkable activity as shown in Table I. α-H_2GTX-II and some of its derivatives (**18**, **27** and **28**) manifested the higher activity than that of natural GTXs.

In this paper, the preparative procedures of rhodojaponin-III (**4**) from GTX-I, 10,14-epoxy-10-deoxy-GTX-III (**6**) from GTX-III, and a number of new derivatives from α-H_2GTX-II are described.

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Conversion of GTX-I into Rhodojaponin-III (**4**) (Scheme 1)

On mild acetylation of GTX-I, 6-*O*-acetyl-GTX-I (**1**) was obtained in quantitative yield. **1** was treated with methanesulfonyl chloride in pyridine to afford Δ^2-6-*O*-acetyl-GTX-I derivative, which was oxidized with *m*-chloroperbenzoic acid in dichloromethane to give a mixture of epoxides, **2** and **3** (7:3). Alkaline hydrolysis of **2** gave **4**, whose IR and PMR spectra and melting point were consistent with those of natural rhodojaponin-III.[6]

10,14-Epoxy-10-deoxy-GTX-III (**6**) (Scheme 2)

Mild acetylation of GTX-III gave 3,6-di-*O*-acetyl-GTX-III (**5**). In order to prepare 14-dehydro-GTX-III, oxidation of **5** with chromic anhydride-pyridine complex followed by alkaline hydrolysis was performed. However, the product was a hemiacetal (**6**), whose IR spectrum showed no absorption due to carbonyl group. In the CMR spectrum of **6**, 14-secondary carbinol carbon signal of GTX-III disappeared and a new quaternary carbon signal bearing two oxygen atoms appeared at δ 114.8. The signal (δ 87.5) of 10-tertiary carbinol shifted to downfield as compared to that (δ 78.1) of GTX-III.[7] An additional support

TABLE I. THE SEQUENCE OF BIOLOGICAL ACTION OF GTX DERIVATIVES

Compound	Critical concentration (M)[b]
2β,3β-Epoxy-2-dehydro-α-H_2GTX-II (**18**)	$3.00\times10^{-6}\pm0$
α-H_2GTX-II	$3.40\times10^{-6}\pm0.27$
Δ^{16}-α-H_2GTX-II (**28**)	$4.00\times10^{-6}\pm0.35$
Δ^{15}-α-H_2GTX-II (**27**)	$4.60\times10^{-6}\pm0.27$
GTX-III[a]	$1.40\times10^{-5}\pm0.27$
Asebotoxin-III[a]	$1.80\times10^{-5}\pm0.22$
Rhodojaponin-III (**4**)	$2.00\times10^{-5}\pm0$
14-Dehydro-α-H_2GTX-II (**23**)	$2.00\times10^{-5}\pm0.28$
2α-Hydroxy-α-H_2GTX-II (**19**)	$2.00\times10^{-5}\pm0$
Asebotoxin-I[a]	$2.20\times10^{-5}\pm0.22$
GTX-I[a]	$3.63\times10^{-5}\pm0.34$
15α-Hydroxy-α-H_2GTX-II (**30**)	$6.00\times10^{-5}\pm0.71$

[a] Natural product.
[b] Details of the bioassay method are described in the previous paper.[5]

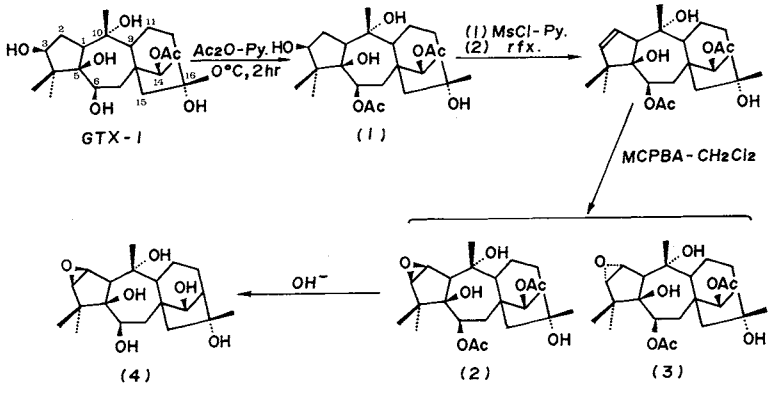
for the structure of **6** was provided by its PMR spectrum, 20-methyl protons signal of **6** appearing at remarkably higher field (δ 1.34) than that of GTX-III (δ 1.86) indicates that the methyl group is not located at or near 1,3-diaxial position to 5β-hydroxyl group owing to the formation of the oxide ring between 10 and 14 position.

3-Dehydro-α-H_2GTX-II (**7**) (Scheme 3)

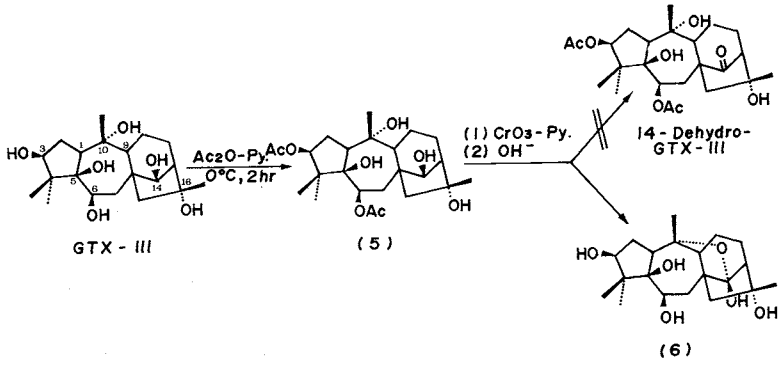
Selective oxidation of α-H_2GTX-II with *N*-bromosuccinimide in aqueous dioxane gave **7**.[8] Its IR spectrum showed an absorption due to carbonyl group (1725 cm^{-1}) and the PMR spectrum are consistent with the proposed structure.

3-Epi-α-H_2GTX-II (**8**) (Scheme 3)

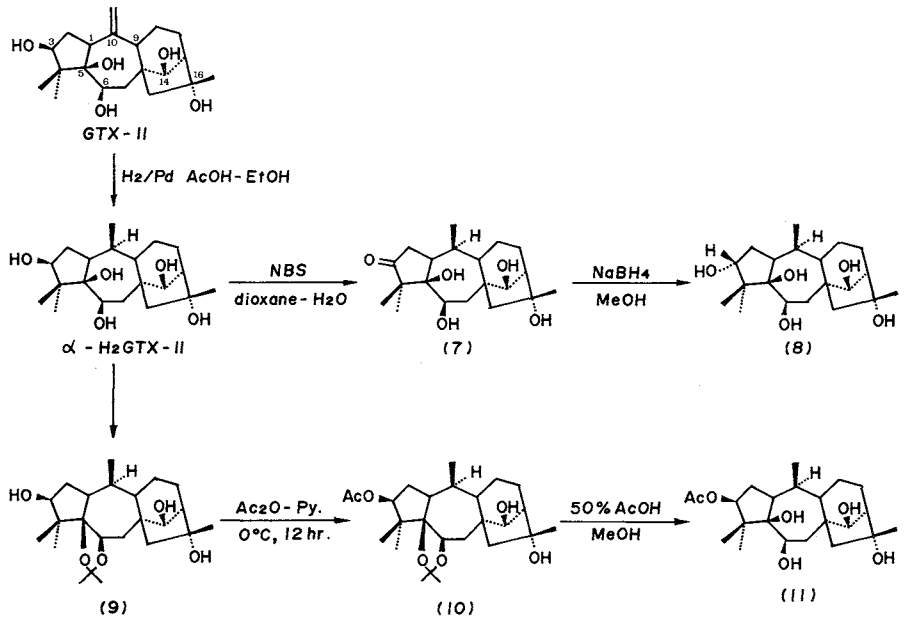
Reduction of **7** with sodium borohydride in



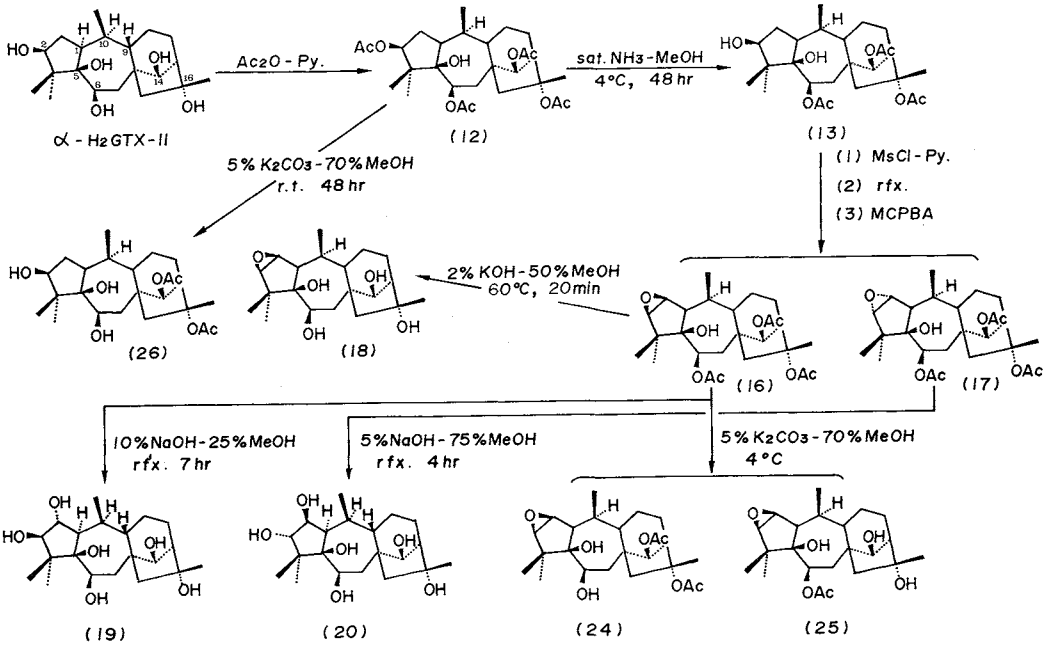
SCHEME 1



SCHEME 2



SCHEME 3



SCHEME 4

methanol afforded **8**. 3-Carbinol proton of **8** resonated at lower field than that of α-H_2GTX-II by 0.62 ppm. This finding indicates that 3-H of **8** has β-configuration.

3-O-Acetyl-α-H_2GTX-II (**11**) (Scheme 3)

Mild acetylation of 5,6-*O,O*-isopropylidene compound (**9**) gave a monoacetate (**10**). Its PMR spectrum showed a singlet due to acetoxy group (δ 2.10), and the acetylation shift

of triplet owing to 3-H ($J=7$ Hz) was also observed. Hydrolysis of **10** with 50% aqueous acetic acid gave **11**.

2β,3β-Epoxy-3-deoxy-α-H_2GTX-II (**18**) (Scheme 4)

Ammonolysis of tetraacetyl-α-H_2GTX-II (**12**) gave a triacetate (**13**), whose PMR spectrum contained three singlets due to acetoxy group. The signal of 3-H of **13** appeared at higher field (δ 3.90) than that of **12** (δ 4.90), whereas the signals of 6-H and 14-H of **13** remained at nearly the same field. According to the same treatment as that employed in the preparation of **2** and **3**, **13** was converted into a mixture of **16** and **17** (7 : 3). Reduction of **16** with lithium aluminum hydride gave α-H_2GTX-II; therefore the epoxy group of **16** should have β-configuration. Alkaline hydrolysis of **16** afforded **18**.

2α-Hydroxy-α-H_2GTX-II (**19**) *and 2β-Hydroxy-3-epi-α-H_2GTX-II* (**20**) (Scheme 4)

Vigorous alkaline hydrolysis of **16** and **17** gave 2,3-*trans*-glycols, (**19**) and (**20**), respectively. The PMR spectrum of **19** showed a double doublet due to 2-H at δ 4.82 ($J=2.5$ and 6.5 Hz) and a doublet due to 3-H at δ 3.95 ($J=2.5$ Hz). The spectrum of **20** showed two doublets ($J=10$ Hz) due to 2-H and 3-H at δ 3.95 and δ 3.68; this finding means that there is no coupling between 2-H and 1-H in **20**. Therefore, 2-H of **20** must have α-configuration.

14-Dehydro-α-H_2GTX-II (**23**) (Scheme 5)

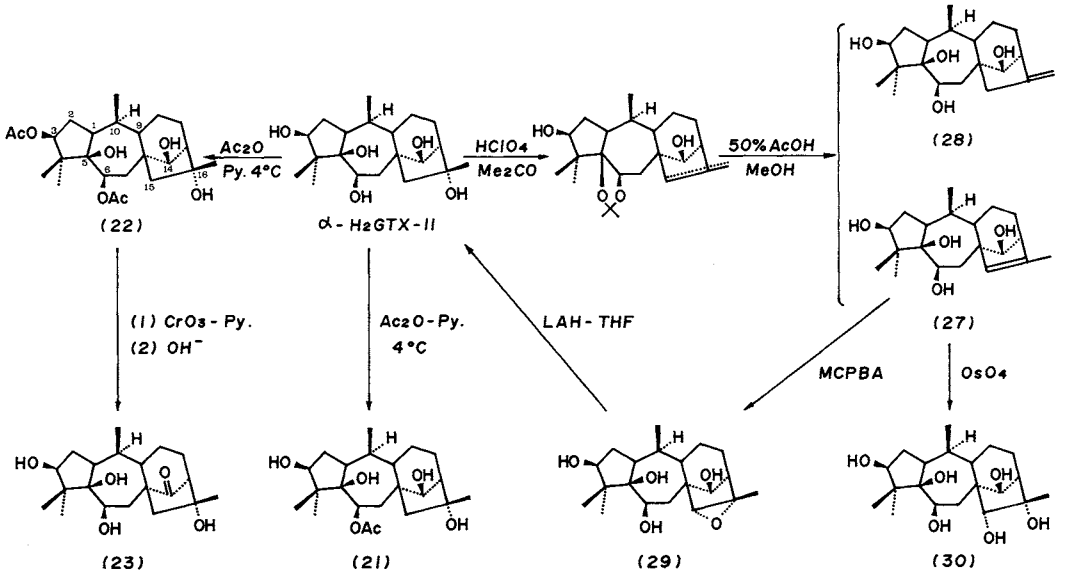
On mild acetylation of α-H_2GTX-II, a mixture of **21** and **22** was obtained. Oxidation of **22** with chromic anhydride–pyridine complex followed by alkaline hydrolysis gave **23**.

2β,3β-Epoxy-14,16-di-O-acetyl-3-deoxy-α-H_2GTX-II (**24**) *and 2β,3β-epoxy-6-O-acetyl-3-deoxy-α-H_2GTX-II* (**25**) (Scheme 4)

Mild alkaline hydrolysis of **16** gave **24** and **25**. The PMR spectrum of **24** contained two singlets due to acetoxy group (δ 1.95 and δ 2.05) and a signal due to 6-H (m. δ 3.55). The PMR spectrum of **25** exhibited only a singlet due to acetoxy group at δ 2.07 and an acetylation shift of 6-H at δ 5.60 (dd, $J=5$ and 10 Hz).

14,16-Di-O-acetyl-α-H_2GTX-II (**26**) (Scheme 4)

Partial hydrolysis of **12** afforded a diacetate (**26**) in addition to the triacetate (**13**). In the PMR spectrum of **26**, one singlet due to 6-



SCHEME 5

acetoxy group was lacked and 6-H signal appeared at remarkable upfield (δ 4.17) as compared to that of **13**.

15α,16α-Epoxy-16-deoxy-α-H_2GTX-II (**29**) (Scheme 5)

The preparation of **27** and **28** was reported previously.[7] Oxidation of **27** with *m*-chloroperbenzoic acid gave **29**. Reduction of **29** with lithium aluminum hydride afforded α-H_2GTX-II; therefore, 15,16-epoxy group of **29** has α-configuration.

15α-Hydroxy-α-H_2GTX-II (**30**) (Scheme 5)

Oxidation of **27** with osmium tetroxide gave **30**, whose PMR spectrum showed a new singlet due to 15-carbinol proton (δ 3.67) as compared to that of α-H_2GTX-II. The structure of **30** was decided by the chemical shift value of 17-methyl protons. If 17-methyl group is located at α-position, the signal should suffer a remarkable downfield shift owing to 1,3-diaxial effect which is caused by 14-hydroxyl group. However, 17-methyl protons appeared at nearly the same field (δ 1.47) as compared to that of α-H_2GTX-II (δ 1.50). This finding indicates that 17-methyl group of **30** has β-configuration and therefore, 15-hydroxyl group has α-configuration.

EXPERIMENTAL

PMR spectra were run on a JEOL JNM-MH-60 spectrometer in pyridine-d_5 using 0.2~0.3 M solutions and tetramethylsilane (TMS) as internal standard, unless otherwise stated. Infrared spectra were recorded on JASCO IRA-2 spectrophotometer. The CMR spectrum of **6** was obtained at 25.05 MHz in the Fourier transform mode on a JEOL FX-100 spectrometer. The sample was examined in pyridine-d_5 solution (0.3 M) with TMS added as internal reference in micro cell at ambient temperature.

Rhodojaponin-III (**4**). To a solution of **1** (150 mg) in pyridine (1 ml) was added 8 drops of methanesulfonyl chloride and the mixture was allowed to stand for 10 hr at −5°C and then refluxed for 2 hr. The mixture was evaporated to dryness and the residue was purified by column chromatography on silica gel. Elution with PhH–EtOAc (50:50) gave **1** (60 mg). The product was dissolved in dichloromethane (2 ml) and *m*-chloroperbenzoic acid (50 mg) was added to this solution, which was kept for 10 hr at room temperature. Excess peracid was decomposed by addition of 10% aq. Na_2SO_3 and the reaction mixture was worked up as usual* to give a crude mixture of **2** and **3**, total 56 mg. These were separated by silica gel TLC (developing solvent: PhH–EtOAc (1:1) into **2** (34 mg) and **3** (14 mg). A solution of **2** (63 mg) in 2% KOH–50% EtOH (5 ml) was warmed at 50°C for 3 hr, neutralized with 0.2 N–HCl and worked up as usual to give a solid, which was recrystallized from EtOAc to yield **4** (38 mg), mp 277°C, IR ν_{max}^{KBr} cm^{-1}: 3500 and 3440 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.27, 1.50, 1.55 and 1.87 (3H, s, CH_3), 3.23 (1H, d, J=2.5 Hz), 4.26 (1H, d, J=2 Hz), 4.48 (1H, dd, J=4.8 and 11.4 Hz, H-6), 4.85 (1H, s, H-14).

10,14-Epoxy-10-deoxy-GTX-III (**6**). Mild acetylation of GTX-III gave **5** in 60% yield. A mixture of **5** (60 mg) in pyridine (1 ml) and CrO_3–Pyr. complex (70 mg in 1 ml) was kept for 20 hr at room temperature. Benzene was added and the organic layer was washed with water. Evaporation of the solvent gave a solid (54 mg). The solution of the solid in MeOH (1 ml) and 2% aq. KOH (1 ml) was heated at 60°C for 1.5 hr. The mixture was diluted with water and worked up as usual to give **6** (36 mg), which was recrystallized from EtOAc. **6**: mp 248.5°C, $[\alpha]_D^{30}$ −42.5° (c=1.2, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.18 (3H, s, Ch_3-18), 1.34 (3H, s, CH_3-20), 1.42 (3H, s, CH_3-17), 1.63 (3H, s, CH_3-19), 3.83 (1H, dd, J=4 and 8 Hz, H-3), 4.86 (1H, t, J=7 Hz, H-6), CMR δ_{TMS}: 20.4 (C-19), 22.1 (C-12), 23.9, 24.1 (C-11, C-17, C-18), 25.1 (C-20), 38.4 (C-2), 42.5 (C-7), 46.8, 56.0, 58.8 (C-1, C-9, C-13), 50.5, 54.5 (C-4, C-8), 71.9 (C-6), 78.7 (C-16), 79.6 (C-3), 86.0 (C-5), 87.5 (C-10), 114.8 (C-14), *Anal.* Found: C, 64.61; H, 9.01. Calcd. for $C_{20}H_{32}O_6$: C, 65.19; H, 8.75%.

3-Dehydro-α-H_2GTX-II (**7**). To a solution of α-H_2GTX-II (200 mg) in dioxane (5 ml) and water (1 ml) was added *N*-bromosuccinimide (NBS) (250 mg) and $CaCO_3$ (200 mg), and left for 40 hr at room temperature. After the treatment of 5% aq. Na_2SO_3 and the reaction mixture was worked up as usual to give an oily product, which was purified with silica gel column. Elution with PhH–EtOAc (30:70) gave **7** (170 mg), which was recrystallized from EtOAc–*n*-hexane: mp 193~4°C, $[\alpha]_D^{30}$ −48.3° (c=1.7, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (C=O), PMR δ_{TMS}: 1.30, 1.46 and 1.55 (3H, s, CH_3), 4.15 (1H, s, H-14), 4.25 (1H, m, H-6), *Anal.* Found: C, 68.02; H, 9.31. Calcd. for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15%.

3-Epi-α-H_2GTX-II (**8**). To a solution of **7** (102 mg) in MeOH was added sodium borohydride ($NaBH_4$) (40 mg) and the mixture was left for 4 hr at room temperature. The reaction mixture was worked up as usual to give a crude

* "Worked up as usual" means extracted with EtOAc and extract was washed, dried over Na_2SO_4, and evaporated under reduced pressure.

crystal, which was recrystallized from EtOAc to give **8** (81 mg); mp 268°C, $[\alpha]_D^{30}$ −10.2° (c=1.3, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.37, 1.48 and 1.58 (3H, s, CH_3), 4.20 (1H, s, H-14), 4.50 (1H, m, H-6), 4.52 (1H, m, H-3), *Anal.* Found: C, 66.99; H, 9.84. Calcd. for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67%.

3-O-Acetyl-α-H_2GTX-II (**11**). A solution of **9** (300 mg) in acetic anhydride (2 ml) and pyridine (2 ml) was kept at 0°C overnight. Working up as usual gave an oily product, which was chromatographed on silica gel. Elution with PhH–EtOAc (70 : 30) gave **10** (220 mg). A solution of **10** (120 mg) in MeOH (2 ml) was boiled with 50% aq. AcOH (2 ml) for 2 hr. The reaction mixture was worked up as usual to give an oil, which was chromatographed on silica gel. Elution with PhH–EtOAc (50 : 50) gave **11** (65 mg), which was recrystallized from EtOAc: mp 178°C, $[\alpha]_D^{30}$ +18° (c=0.56, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1730 (AcO), PMR δ_{TMS}: 1.25, 1.47 and 1.50 (3H, s, CH_3), 2.07 (AcO), 4.33 (1H, s, H-14), 4.47 (1H, dd, J=5 and 12Hz, H-6), 5.03 (1H, t, J=3 Hz, H-3), *Anal.* Found: C, 66.45; H, 9.37. Calcd. for $C_{22}H_{36}O_6$: C, 66.64; H, 9.15%.

2β,3β-Epoxy-3-deoxy-α-H_2GTX-II (**18**). A solution of **12** (100 mg) in MeOH saturated with NH_3 (10 ml) was kept at 4°C for 48 hr. Evaporation of the solvent gave an oily product, which was purified with silica gel column. Elution with PhH–EtOAc (80 : 20) gave **13** (70 mg). The mixture of **16** and **17** (total 48 mg) was obtained from **13** (100 mg) by the procedure for **2** and **3**. The mixture (48 mg) was separated by silica gel column. Elution with PhH–EtOAc (90 : 10) and succesively with PhH–EtOAc (85 : 15) gave **17** (11 mg) and **16** (28 mg), respectively. A mixture of **16** (65 mg) in MeOH (1 ml) and 2% aq. KOH (1 ml) was stirred at 60°C for 20 min. The reaction mixture was worked up as usual to give a solid, which was recrystallized from EtOAc to yield **18** (32 mg); 221 ~ 22°C, $[\alpha]_D^{30}$ −51.8° (c=1.0, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), PMR δ_{TMS}: 1.23, 1.48 and 1.53 (3H, s, CH_3), 3.23 (1H, d, J=3.2 Hz, H-2 or H-3), 3.57 (1H, d, J=3.2 Hz, H-3 or H-2), 4.27 (1H, s, H-14), 4.33 (1H, m, H-6), *Anal.* Found: C, 67.99; H, 9.37. Calcd. for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15%.

2α-Hydroxy-α-H_2GTX-II (**19**) *and 2β-Hydroxy-3-epi-α-H_2GTX-II* (**20**). A mixture of **16** (100 mg) in MeOH (1 ml) and 10% aq. NaOH (3 ml) was refluxed for 7 hr. The reaction mixture was worked up as usual to give the crude **19**, which was purified by silica gel thin layer chromatography (TLC) (developing solvent: EtOAc) to give pure **19** (20 mg), which was recrystallized from EtOAc; mp 250°C, $[\alpha]_D^{30}$ −24.7° (c=0.57, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.47 (3H, s, CH_3), 1.63 (6H, s, CH_3), 3.95 (1H, d, J=2.5 Hz, H-3), 4.38 (1H, s, H-14), 4.50 (1H, dd, J=5 and 10 Hz, H-6), 4.82 (1H, dd, J=2.5 and 6.5 Hz, H-2), *Anal.* Found: C, 64.37; H, 9.47. Calcd. for $C_{20}H_{34}O_6$: C, 64.84; H, 9.25%.

A mixture of **17** (80 mg) in MeOH (3 ml) and 5% aq. NaOH (1 ml) was refluxed for 4 hr. Working up as usual gave a solid, which was recrystallized from EtOAc to give **20** (34 mg); mp 215°C, $[\alpha]_D^{30}$ −23.5° (c=0.8, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.10, 1.20 and 1.47 (3H, s, CH_3), 3.68 (1H, d, J=10 Hz), 3.95 (1H, d, J=10 Hz), 4.23 (1H, s, H-14), 4.50 (1H, dd, J=5 and 10 Hz, H-6), *Anal.* Found: C, 64.48; H, 9.48. Calcd. for $C_{20}H_{34}O_6$: C, 64.84; H, 9.25%.

14-Dehydro-αH_2GTX-II (**23**). A mixture of α-H_2GTx-II (228 mg) in acetic anhydride (2 ml) and pyridine (2 ml) was kept at 4°C for 90 hr. The reaction mixture was worked up as usual to give an oily product, which was separated into three fractions with silica gel TLC (developing solvent: PhH–EtOAc (1 : 2)). From the lower fraction **21** (60 mg) was obtained, and **22** (84 mg) was isolated from the middle fraction. To a solution of **22** (120 mg) in pyridine (1 ml), CrO_3–pyridine complex (200 mg in 2 ml) was added and the mixture was left for 6 hr at room temperature. Working up as usual gave a solid (110 mg). A mixture of the solid (110 mg) in MeOH (1 ml) and 4% aq. K_2CO_3–70% MeOH (10 ml) was stirred at room temperature for 30 hr. After removal of MeOH *in vacuo*, the residue was worked up as usual to give a solid, which was purified with silica gel TLC (developing solvent: EtOAc) to yield **23** (80 mg). **21** and **23** were recrystallized from EtOAc.

21: mp 229°C, $[\alpha]_D^{30}$ +7.5° (c=0.54, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 1730 (AcO), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 0.88 (1H, s, CH_3), 1.49 (6H, s, CH_3), 2.07 (3H, s, AcO), 3.92 (1H, m, H-3), 4.35 (1H, s, H-14), 5.60 (1H, dd, J=5 and 10 Hz, H-6), *Anal.* Found: C, 65.86; H, 9.40. Calcd. for $C_{22}H_{36}O_6$: C, 66.64; H, 9.15%.

23: mp 203°C, $[\alpha]_D^{30}$ −4° (c=1.25, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1718 (C=O), PMR δ_{TMS}: 1.25, 1.55 and 1.63 (3H, s, CH_3), 3.28 (1H, m, H-13), 3.98 (1H, m, H-3), 5.08 (1H, t, J=8 Hz, H-6), *Anal.* Found: C, 67.77; H, 9.35. Calcd. for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15%.

2β,3β-Epoxy-14,16-di-O-acetyl-3-deoxy-α-H_2GTX-II (**24**) *and 2β,3β-Epoxy-6-O-acetyl-3-deoxy-α-H_2GTX-II* (**25**). A mixture of **16** (200 mg) in MeOH (3 ml) and 5% aq. K_2CO_3–70% MeOH (20 ml) was kept at 4°C for 6 days. After removal of MeOH *in vacuo*, the residue was worked up as usual to give an oily product, which was chromatographed on silica gel. Elution with PhH–EtOAc (70 : 30) and successively with PhH–EtOAc (50 : 50) gave **24** (34 mg) and **25** (16 mg), respectively, the former was recrystallized from EtOAc–*n*-hexane and the latter from EtOAc.

24: mp 188°C, $[\alpha]_D^{27}$ −25.4° (c=0.7, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (AcO), PMR $\delta_{TMS}^{CDCl_3}$: 1.05, 1.20 and 1.60 (3H, s, CH_3), 1.25 (3H, d, J=7 Hz, CH_3-20), 1.93 and 2.02 (3H, s, AcO), 3.13 and 3.45 (1H, d, J=3 Hz, H-2 and H-3), 3.47 (1H, dd, J=5 and 10 Hz, H-6), 4.98 (1H, s, H-14), *Anal.* Found: C, 65.61; H, 8.53. Calcd. for $C_{24}H_{36}O_7$: C, 66.03; H, 8.31%.

25: mp 270°C (dec.), $[\alpha]_D^{20}$ −38.6° (c=0.52, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3560 and 3450 (OH), 1730 (AcO), PMR δ_{TMS}:

0.93, 1.40 and 1.48 (3H, s, CH_3), 2.03 (3H, s, AcO), 3.25 and 3.57 (1H, d, $J=3$ Hz, H-2 and H-3), 4.20 (1H, s, H-14), 5.42 (1H, m, H-6), *Anal.* Found: C, 65.83; H, 8.77. Calcd. for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69%.

14,16-Di-O-acetyl-α-H_2GTX-II (**26**). A mixture of **12** (120 mg) in MeOH (3 ml) and 5% aq. K_2CO_3–70% MeOH (20 ml) was kept at room temperature for 48 hr. After removal of MeOH *in vacuo*, the reaction mixture was worked up as usual to give a mixture of **13** and **26**, which was separated by silica gel column. Elution with PhH–EtOAc (70:30) and successively with PhH–EtOAc (60:40) gave **13** (45 mg) and **26** (42 mg), respectively. **26** was recrystallized from EtOAc–*n*-hexane.

26: mp 195°C, $[\alpha]_D^{30}$ +8.3° ($c=1.2$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1730 (AcO), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.27 (3H, s, CH_3), 1.73 (6H, s, CH_3), 2.14 and 2.23 (3H, s, AcO), 4.10 (1H, m, H-3), 4.17 (1H, m, H-6), 5.65 (1H, s, H-14), *Anal.* Found: C, 65.75; H, 8.94. Calcd. for $C_{24}H_{38}O_7$: C, 65.73, H, 8.73%.

15α,16α-Epoxy-16-deoxy-α-H_2GTX-II (**29**). *m*-Chloroperbenzoic acid (80 mg) was added to a solution of Δ^{15}-α-H_2GTX-II (**27**) (100 mg) in dichloromethane (2 ml). The mixture was left overnight at room temperature. Excess peracid was decomposed by addition of 10% aq. Na_2SO_3, and the reaction mixture was worked up as usual to give crude crystal, which was recrystallized from EtOAc to afford **29** (80 mg); mp 234°C, $[\alpha]_D^{30}$ +48.6° ($c=0.7$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.17, 1.38 and 1.67 (3H, s, CH_3), 2.97 (1H, s, H-15), 3.77 (1H, s, H-14), 3.87 (1H, m, H-3), 4.37 (1H, dd, $J=5$ and 10 Hz, H-6), *Anal.* Found: C, 68.25; H, 9.43. Calcd. for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15%.

15α-Hydroxy-α-H_2GTX-II (**30**). A mixture of **27** (200 mg) and osmium tetroxide (150 mg) in pyridine (2 ml) was stirred for 2 hr at room temperature, and a solution of Na_2SO_3 (300 mg) in water (5 ml) and pyridine (4 ml) was added with stirring. After 5 min, the reaction mixture was extracted with EtOAc and the extract was evaporated to dryness. The residue was chromatographed on silica gel column. Elution with PhH–EtOAc (10:90) gave **30** (50 mg), which was recrystallized from EtOAc; mp 136~38°C (dec.), $[\alpha]_D^{30}$ +16.2° ($c=0.74$, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), PMR $\delta_{TMS}^{Py\text{-}d_5+D_2O}$: 1.13 (3H, s, CH_3-18), 1.47 (3H, s, CH_3-17), 1.60 (3H, s, CH_3-19), 3.67 (1H, s, H-15), 3.77 (1H, m, H-3), 4.15 (1H, s, H-14), 4.40 (1H, dd, $J=5$ and 10 Hz, H-6), *Anal.* Found: C, 64.03; H, 9.53. Calcd. for $C_{20}H_{34}O_6$: C, 64.84; H, 9.25%.

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