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GRAYANOTOXIN-XVIII AND GRAYANOSIDE B, A NEW A-NOR-B-HOMO-ENT-KAURENE AND ITS GLUCOSIDE FROM *LEUCOTHOE GRAYANA*

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Key Word Index—*Leucothoe grayana*; Ericaceae; diterpenoid; diterpenoid glucoside; grayanotoxin-XVIII; grayanoside B.

Abstract—From *Leucothoe grayana* a new grayanoid (diterpene) and its glucoside have been isolated. The structures of these new natural compounds have been established by chemical and spectroscopic means and by correlation with a known compound.

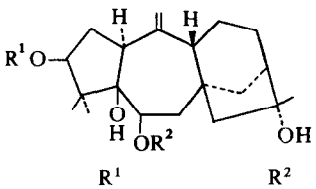
INTRODUCTION

In a previous paper [1] we reported the isolation of the first grayanoid glucoside, grayanoside A, from *Leucothoe grayana*. Grayanoside B and grayanotoxin-XVIII have now been isolated from a methanolic extract of the same plant. Grayanoside B is more polar than grayanoside A on Si gel TLC. Grayanotoxin XVIII is a new grayanoid.

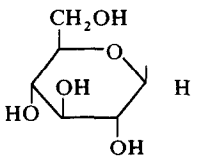
RESULTS AND DISCUSSION

Grayanoside B (**1a**), viscous syrup, was obtained from the *n*-BuOH soluble fraction of an MeOH extract. Its PMR and ^{13}C NMR spectra disclosed the presence of many OH groups, three Me groups and a vinylidene group. Acetylation of **1a** with Ac_2O–Py gave a pentaacetate (**1b**). Its IR spectrum still showed an OH group at 3525 cm^{-1}, but it could not be oxidized with CrO_3–Py. This result suggested that **1a** may have a tertiary OH group.

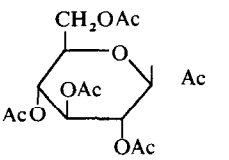
Acid hydrolysis of **1a** yielded glucose, but its aglycone could not be obtained. Enzymatic hydrolysis of **1a** with naringinase gave a genuine aglycone (**2a**), mp 162–164°, whose molecular formula was shown to be $C_{20}H_{32}O_4$ by means of high resolution MS. PMR and ^{13}C NMR spectra revealed the presence of three Me groups, two carbinyl protons, four carbinyl carbons and one vinylidene group. Acetylation of **2a** gave a diacetate (**2b**), whose IR spectrum still showed an OH group. These data suggested that **1a** is a new grayanoid with the structure shown. In order to confirm this structural assignment grayanotoxin-I (**3**) was treated with Me_2CO/*p*-toluenesulfonic acid to give an acetonide (**4**), which was then acetylated to furnish a diacetate (**5**). Partial hydrolysis of **5** afforded a monoacetate (**6**) and subsequent oxidation of **6** with Jones' reagent gave a ketone (**7**). The Wolff–Kishner reduction of **7** afforded 14-deoxygrayanotoxin (**8**), which was acetylated again and then dehydrated with thionyl-chloride–Py to give 3,16-diacetyl-5,6-*O*-isopropylidene-14-deoxygrayanotoxin-II (**10**). On the other hand, **2a** yielded an acetonide (**11**) on treatment with Me_2CO/*p*-toluenesulfonic acid and subsequent acetylation gave an acetonide acetate, which was in all respects identical with the above deoxygrayanotoxin II (**10**).



R^1 R^2



1a H



1b Ac

2a H H

2b Ac Ac

In the PMR spectra the anomeric proton of **1a** and **1b** was observed at δ 4.90, *d*, J = 8 Hz and δ 4.48, *d*, J = 8 Hz, respectively. Therefore, **1a** must be a β-D-glucoside. We determined the glycosidation position in the aglycone (**2a**) by ^{13}C NMR spectroscopy. The ^{13}C NMR signals were assigned by means of single-frequency off-resonance decoupling, selective proton decoupling and by comparing the spectra of pairs of compounds. In general, carbinyl carbon (α-carbon) signals of aglycone alcohols are displaced by +7.0 ppm on glucosidation [2, 3]. The C-3 signal of the aglycone (**2a**) was observed at δ 81.2, while that of the glucoside (**1a**) was observed at δ 88.6. On the other hand, other carbinyl carbon signals scarcely shifted

on glucosidation. From these data, β-D-glucose must be bound at C-3 of the aglycone (**2a**).

From the above data, grayanoside B (**1a**) has been concluded to be 5β,6β,16α-trihydroxy-3β-(β-D-glucopyranosyl)oxy-A-nor-B-homo-ent-kaur-10(20)-ene.

A new grayanoid was isolated from the $CHCl_3$ fraction and EtOAc fraction of a MeOH extract of the same plant by very careful chromatography. It was identified with the aglycone (**2a**) of grayanoside B by comparison of TLC, PMR and IR. Since it was obtained from a natural source, we named it grayanotoxin-XVIII.

EXPERIMENTAL

Mps were uncorr. PMR spectra were measured at 100 MHz. ^{13}C NMR spectra were measured at 15 MHz. The δ values are in ppm downfield from TMS as an internal standard. Plants were collected at Hokkaido (northern island of Japan).

Extraction and isolation of **1a**. Air-dried leaves and stems (4.3 kg) were extracted, under reflux, with C_6H_6 and MeOH, successively. The methanolic extracts were diluted with 3 l. of H_2O. The ppt. was filtered off and then satd lead subacetate soln was added to the filtrate. The resulting ppt. was filtered and then H_2S gas bubbled into the filtrate and the ppt. (PbS) was separated. The soln was concd *in vacuo* to 700 ml, and extracted with $CHCl_3$, EtOAc and *n*-BuOH, successively. The *n*-BuOH fraction was chromatographed on a column of activated charcoal. MeOH–H_2O (70:30 to 80:20) eluate was concd to dryness and chromatographed on a Si gel column. EtOH–EtOAc (1:99) eluate gave crude **1a** (4 g), which was purified on a silanized Si gel column in MeOH–H_2O (20:80 to 40:60).

Grayanoside B (**1a**). Viscous syrup. $[\alpha]_D^{16}$ −14.1° (MeOH c = 2.55), HPLC: (JASCO FLC-150) gradient: 20% MeOH to 26% MeOH in H_2O at 1.5%/min, flow rate: 0.75 ml/min, column: 50 cm × 2.1 mm φ, JASCODAC SV-02, detector: UV detector operating at 204 nm (JASCO UVIDEC 100), R_t: 8.8 min. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1075, 1033. PMR (C_5D_5N): δ 1.28, 1.52, 1.72 (each 3H, *s*), 3.8–4.6 (many protons), 4.90 (1H, *d*, J = 8 Hz), 5.02 (1H, *s*), 5.07 (1H, *s*). ^{13}C NMR (C_5D_5N): δ 20.1 (CH_3), 23.8 ($—CH_2—$), 25.6 (CH_3), 26.3 ($—CH_2—$), 26.9 (CH_3), 35.7 ($—CH_2—$), 37.5 ($—CH_2—$), 43.1 ($—\underset{|}{C}H$), 44.4 ($—\underset{|}{\underset{|}{C}}—$), 46.5 ($—CH_2—$), 47.6 ($—\underset{|}{C}H$), 50.6 ($—\underset{|}{\underset{|}{C}}—$), 54.2 ($—\underset{|}{C}H$), 62.8 ($—CH_2—$ and glucose C-6), 71.5 [$—CHOH$(C-6) and glucose C-4], 75.4 (glucose C-2), 78.3 (glucose C-3, C-5), 79.3 [$—\underset{|}{C}OH$ (C-16)], 82.3 [$—\underset{|}{C}OH$ (C-5)], 88.6 [$—CHO—$ (C-3)], 105.4 (glucose C-1), 112.7 ($C=\underline{C}H_2$), 151.6 ($\underline{C}=CH_2$).

Pentaacetylgrayanoside B (**1b**). Treatment of **1a** with Ac_2O–Py for 19 hr at room temp. gave **1b**: mp 212–214° (*iso*-PrOH). (Found: C, 61.03; H, 7.80. Calcd for $C_{36}H_{52}O_{14}$: C, 61.00; H, 7.40%). IR ν_{max}^{KBr} cm^{-1}: 3525, 1755, 1378, 1230, 1035. PMR ($CDCl_3$): δ 0.95, 1.03, 1.36 (each 3H, *s*), 2.00 (3H × 5, *s*), 3.60 (2H, *m*), 4.20 (2H, *m*), 4.48 (1H, *d*, J = 8 Hz), *ca* 5.0 (6H, *m*).

Oxidation of **1b**. CrO_3 was added to a Py soln of **1b** and then the reaction mixture was stirred at room temp. for 6 days. However, **1b** was recovered quantitatively.

Acid hydrolysis of **1a**. A soln of **1a** (4 mg) in dioxane (1 ml) and 5% H_2SO_4 (2 ml) was heated for 3.5 hr on a steam bath. The mixture was cooled, diluted with H_2O (2 ml) and extracted with EtOAc. The EtOAc extract was evapd *in vacuo* to give a complex mixture. The aq. layer was treated with Amberlite CG-4B (OH^-) and evapd *in vacuo* to give the sugar moiety. The sugar was converted to its TMSi derivative and identification was made by comparison of R_t of authentic TMSi-D-glucose by GLC. GLC was performed at 171°, using FID, on a stainless column (2 m × 3 mm) of 5% OV-1 on Chromosorb W(AW).

Enzymatic hydrolysis of **1a**. To a soln of **1a** (114 mg) dissolved in HOAc–NaOAc buffer (pH 4.1, 20 ml) crude naringinase 'SANKYO' (250 mg) was added and the reaction mixture was incubated for 47 hr at 37°. It was extracted with EtOAc and purified by Si gel PLC (eluent: EtOH–$CHCl_3$; 1:9) to give aglycone (**2a**) 39 mg, recrystallized from *iso*-propyl ether. Mp 162–164°. $[\alpha]_D^{25}$ −6.81° (MeOH c = 2.20). High resolution MS (75 eV, direct inlet): 336.231, required for $C_{20}H_{32}O_4$: 336.235. IR ν_{max}^{KBr} cm^{-1}: 3350, 1628, 1377, 1030. PMR ($CDCl_3$): δ 1.02, 1.24, 1.36 (each 3H, *s*), 3.60 (1H, *d*, J = 6 Hz), 3.76 (1H, *dd*, J = 3, 11 Hz), 4.95 (1H, *s*), 5.09 (1H, *s*). ^{13}C NMR (C_5D_5N): δ 19.2 (CH_3), 24.1 (CH_3, $—CH_2—$), 25.4 (CH_3), 25.8 ($—CH_2—$), 36.5 ($—CH_2—$), 39.4 ($—CH_2—$), 44.5 ($—\underset{|}{C}H$), 44.7 ($—\underset{|}{C}—$), 46.7 ($—CH_2—$), 47.9 ($—\underset{|}{C}H$), 50.6 ($—\underset{|}{C}—$), 52.3 ($—\underset{|}{C}H$), 62.5 ($—CH_2—$), 70.6 [$—\underset{|}{C}HOH$ (C-6)], 79.5 [$—\underset{|}{C}OH$ (C-16)], 81.2 [$—\underset{|}{C}HOH$ (C-3)], 83.5 [$—\underset{|}{C}OH$ (C-5)], 112.2 ($C=\underline{C}H_2$), 153.0 ($\underline{C}=CH_2$).

Acetylation of **2a**. Treatment of **2a** with Ac_2O–Py for 18 hr at room temp. gave a diacetate **2b**: mp 120–122° (*n*-hexane–Et_2O). (Found: C, 68.36; H, 8.70. Calcd for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63%). IR ν_{max}^{KBr} cm^{-1}: 3510, 3300, 1730, 1630, 1380, 1255, 1032. PMR ($CDCl_3$): δ 1.03 (6H, *s*), 1.36 (3H, *s*), 2.05 (6H, *s*), 4.91 (3H, *s* and *m*), 5.04 (1H, *s*).

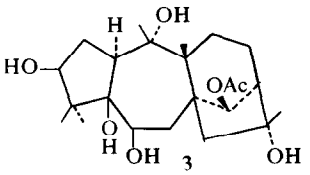
5,6-O-*Isopropylidenegrayanotoxin-I* (**4**). *p*-Toluenesulfonic acid (1 g) was added to a suspension of grayanotoxin-I (1 g) in a small amount of MeOH–dried Me_2CO (80 ml) mixture at 0°. The reaction mixture was stirred for 6.5 hr at 0°, and then was poured into K_2CO_3 soln. Organic solvents were removed from the soln *in vacuo* and the remaining aq. soln was extracted with EtOAc. The extract was washed with H_2O and removal of the solvent *in vacuo* gave **4** (860 mg). Mp 176–178° (EtOAc). (Found: C, 66.11; H, 8.74. Calcd for $C_{25}H_{40}O_7$: C, 66.34; H, 8.91%). IR ν_{max}^{KBr} cm^{-1}: 3460, 1710, 1380, 1260. PMR ($CDCl_3$): δ 0.88, 1.08 (each 3H, *s*), 1.38 (6H, *s*), 1.45, 1.52, 2.15 (each 3H, *s*), 3.65 (1H, *m*, C-3-H), 4.28 (1H, *br d*, J = 4 Hz, C-6-H), 5.85 (1H, *s*, C-14-H).

3-*Acetyl*-5,6-O-*isopropylidenegrayanotoxin-I* (**5**). Treatment of **4** (253 mg) with Ac_2O–Py overnight at room temp. gave **5** (273 mg). Mp 243–244° (EtOAc, lit. [4] mp 220–221°). (Found: C, 65.73; H, 8.70. Calcd for $C_{27}H_{42}O_8$: C, 65.56; H, 8.56%). IR ν_{max}^{KBr} cm^{-1}: 3550, 1748, 1733. PMR ($CDCl_3$): δ 0.91, 0.95 (each 3H, *s*), 1.34 (6H, *s*), 1.38, 1.47, 2.06, 2.10 (each 3H, *s*), 4.20 (1H, *br d*, J = 4 Hz, C-6-H), 4.81 (1H, *t*, J = 7 Hz, C-3-H), 5.87 (1H, *s*, C-14-H).

3-*Acetyl*-5,6-O-*isopropylidenegrayanotoxin-III* (**6**). A soln of **5** (196 mg) in 1% K_2CO_3–MeOH was left for 45 min at room temp. The reaction mixture was neutralized with 1% HCl, then evapd *in vacuo*. The residue was added into H_2O and extracted with EtOAc. The extract was evapd, purified on a Si gel column (eluent: $CHCl_3$–MeOH; 97:3) to yield **6** (145 mg). Mp 202–204° (EtOAc). (Found: C, 66.09; H, 8.76. Calcd for $C_{25}H_{40}O_7$: C, 66.34; H, 8.91%). IR ν_{max}^{KBr} cm^{-1}: 3450, 1732. PMR (C_5D_5N): δ 0.89, 1.03, 1.37, 1.54 1.57, 1.64, 2.00 (each 3H, *s*), 4.40 (1H, *d*, J = 5 Hz, C-6-H), 4.92 (1H, *t*, J = 7 Hz, C-3-H), 5.10 (1H, *s*, C-14,H).

3-*Acetyl* 5,6-O-*isopropylidene*-14-*ketograyanotoxin-III* (**7**). An Me_2CO soln of **6** (180 mg) was treated with Jones' reagent [5]. The reaction mixture was diluted with H_2O and extracted with Et_2O. The extract was evapd, purified on a Si gel column (eluent $CHCl_3$–MeOH, 99:1) to yield **7** (137 mg). Mp 192–195 (diisopropyl ether). (Found: C, 65.54; H, 8.20. Calcd for $C_{25}H_{38}O_7 \cdot \frac{1}{2}H_2O$: C, 65.34; H, 8.55%). IR ν_{max}^{KBr} cm^{-1}: 3462, 3512, 3475, 1733, 1712. PMR ($CDCl_3$): δ 0.94, 1.10, 1.31, 1.35, 1.45, 1.49, 2.06 (each 3H, *s*), 4.29 (1H, *d*, J = 6 Hz, C-6-H), 4.81 (1H, *t*, J = 6 Hz, C-3-H).

5,6-O-*Isopropylidene*-14-*deoxygrayanotoxin-III* (**8**). A mixture of **7** (157 mg), $NH_2NH_2 \cdot H_2O$ (6 ml) and $NH_2NH_2 \cdot 2HCl$ (310 mg) in ethyleneglycol (15 ml) was heated for 4 hr at 130°. Then, KOH (1.6 g) was added and heated again for 10 hr



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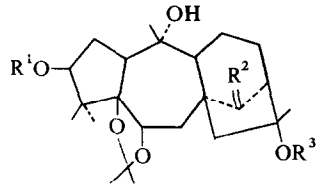
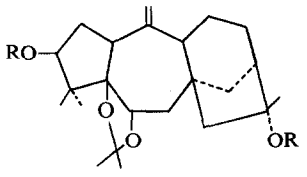


	R^1	R^2	R^3
4	H	OAC, H	H
5	Ac	OAc, H	H
6	Ac	OH, H	H
7	Ac	=O	H
8	Ac	H, H	H
9	Ac	H, H	Ac



	R
10	Ac
11	H

at 210°. After cooling, the mixture was poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and evapd. The residue was purified on a Si gel column (eluent $CHCl_3$–MeOH, 19:1) to give **8** (120 mg). IR ν_{max}^{KBr} cm^{-1}: 3410, no absorbance of C=O. PMR ($CDCl_3$): δ 0.89, 1.09 (each 3H, *s*), 1.37 (6H, *s*), 1.43, 1.50 (each 3H, *s*), 3.60 (1H, *m*, C-3-H), 4.23 (1H, *d*, J = 6 Hz, C-6-H).

3,16 - *Diacetyl* - 5,6 - O - *isopropylidene* - 14 - *deoxygraya* - *notoxin-III* (**9**). A mixture of **8** (120 mg), Py (1 ml) and Ac_2O (1 ml) was heated for 40 hr at 90°. After cooling, the reaction mixture was poured into H_2O and extracted with Et_2O. The extract was evapd, purified on a Si gel column (eluent $CHCl_3$–MeOH, 49:1) to yield **9** (106 mg). Mp 172–174° (Et_2O). (Found: C, 67.84; H, 9.00. Calcd for $C_{27}H_{42}O_7$: C, 67.75; H, 8.85%). IR ν_{max}^{KBr} cm^{-1}: 3485, 1728, 1712. PMR ($CDCl_3$): δ 0.92, 0.99, 1.32, 1.37, 1.47, 1.60, 1.96, 2.07 (each 3H, *s*), 4.17 (1H, *d*, J = 6 Hz, C-6-H), 4.80 (1H, *t*, J = 6 Hz, C-3-H).

3,16 - *Diacetyl* - 5,6 - O - *isopropylidene* - 14 - *deoxygraya* - *notoxin-II* (**10**). A few drops of $SOCl_2$ was added to a Py soln (2 ml) of **9** (73 mg) under cooling. After 5 min, the reaction mixture was poured into H_2O and extracted with Et_2O. The extract was evapd and purified by Si gel PLC (1 mm, eluent C_6H_6–EtOAc, 9:1, detection: I_2 vapor) to give **10** (20 mg) an amorphous powder. High resolution MS (70 eV, direct inlet): 460.288; required for $C_{27}H_{40}O_6$: 460.282. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1642, no absorption band of OH. PMR ($CDCl_3$): δ 0.95, 1.02, 1.32, 1.40, 1.62, 1.98, 2.08 (each 3H, *s*), 3.37 (1H, *m*), 4.17 (1H, *br d*, J = 5 Hz, C-6-H), 4.88 (1H, *dd*, J = 7, 8 Hz, C-3-H), 5.00, 5.14 (each 1H, *br s*, C-20-H).

Acetonation of **2a**. A soln of **2a** (39 mg) and *p*-toluenesulfonic acid (200 mg) in dried Me_2CO (25 ml) was left in a refrigerator for 2 days, and poured into 1N Na_2CO_3 and then extracted with CH_2Cl_2. The extract was evapd, purified on a Si gel column (eluent $CHCl_3$–CH_3CN, 94:6) to give an acetonide (**11**). PMR ($CDCl_3$): δ 0.90, 1.08, 1.36 (each 3H, *s*), 1.38 (6H, *s*), 1.42 (3H, *s*), 3.60 (1H, *m*, C-3-H), 4.24 (1H, *d*, J = 5 Hz, C-6-H), 5.02, 5.14 (each 1H, *brs*, C-20-H).

Acetylation of **11**. Treatment of **11** (40 mg) with Ac_2O–Py for 40 hr at 90° gave an acetonide acetate (**12**). **12** was identified with **10** by comparison of PMR, high resolution MS, TLC and IR.

Isolation of grayanotoxin-XVIII. The $CHCl_3$ fraction and the EtOAc fraction described above were chromatographed on a Si gel column. The eluate of C_6H_6–EtOAc (1:1) was separated from grayanotoxin-IV and chlorophyll by Si gel PLC (MeOH–$CHCl_3$, 1:9) and separated from grayanotoxin-XIV by silanized Si gel PLC (MeOH–H_2O; 1:1) to yield a new diterpene. It was purified with Si gel PLC (MeOH–$CHCl_3$, 1:9). This compound was identified with the aglycone (**2a**) of grayanoside B by comparison of TLC, PMR, IR and MS.

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