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New A-*nor*-B-*homo*-*ent*-Kauranoids (Grayanotoxin XVI and XVII) from *Leucothoe Grayana Max.*

Shinsei GASA, Ryūzō IKEDA, Nobuyuki HAMANAKA, and Takeshi MATSUMOTO
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060

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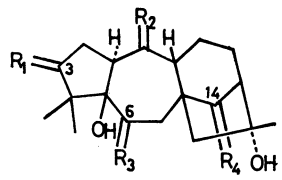
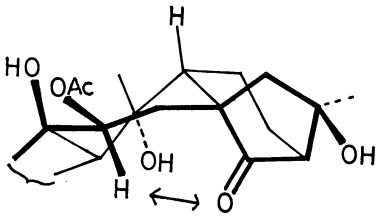
Synopsis. Two physiologically active diterpenoids, grayanotoxin XVI and XVII, have been isolated from *Leucothoe grayana Max.*, and the structures have been determined to be 6-*O*-acetylgrayanotoxin II and 3,6-didehydrograyanotoxin III respectively on the basis of spectral and chemical evidence.

The structures of a number of diterpenoids with an A-*nor*-B-*homo*-*ent*-kaurane skeleton have been elucidated by several groups.[1] In this paper we wish to report on the isolation and characterization of two physiologically active diterpenoids of this type from *L. grayana Max.* These toxic substances, grayanotoxins (hereafter G) XVI and XVII, were obtained as minor constituents by the careful chromatography of alcohol extracts of dried leaves, together with G I,[1] II,[1] III,[1] IV,[2] V,[2] XIV,[3], XV,[3] as well as leucothols A, [4a] B[4b] and C.[4b]

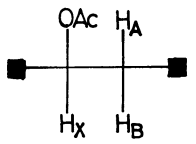
The first compound, G XVI ($C_{22}H_{34}O_6$, mp 116—118 °C, $[\alpha]_D$ +2.8 ° (*c* 1, MeOH) was obtained in a 5×10^{-6}% yield from dried leaves. The IR and NMR spectra indicated the presence of the following groups: two tertiary methyls (δ 0.92 and 1.20), a tertiary methyl on a carbon bearing a hydroxyl group (δ 1.33), two secondary hydroxyls (3511 cm^{-1}, δ 3.57, 1H, q, J_{AX+BX}=3+6 Hz and 4.44, 1H, s), a secondary acetoxyl (1719 cm^{-1}, δ 2.12, 3H, s, and 4.77, 1H, q, J_{AX+BX}= 2+10 Hz), and an exocyclic methylene group (1634 cm^{-1}, δ 5.00 and 5.16, each 1H, s). Since the treatment of this compound with sodium hydroxide in ethanol afforded G II **1**, the new toxin must be either 3-*O*- or 6-*O*-acetyl G III. In order to clarify the position of the acetoxyl group, the toxin was next oxidized with chromium trioxide in pyridine to give an amorphous diketone **7**, whose NMR spectrum indicated a quartet (J_{AX+BX}=4+9 Hz) due to the acetoxyl-bearing methine proton at δ 6.39. The extraordinary large downfield shift of the quartet indicates the steric closeness of the methine proton and the newly-formed carbonyl group (s). Since, on the other hand, **7** seemingly takes a G I-like conformation, **8**,[5] the 6-*O*-acetyl structure was concluded for the diketone and, accordingly, for G XVI also. Supporting evidence for the **2** structure was obtained by means of the INDOR technique. On monitoring the four lines due to the C-6 proton under INDOR conditions, only eight INDOR peaks altogether were observed at δ 2.76. 2.59, 2.52, 2.35, 2.12, 2.08, 1.88, and 1.84, indicating the presence of a **9** moiety (δ_A2.01, δ_B2.53, J_{AB}=−14.4 Hz, J_{AX}= 2.4 Hz, J_{BX}=10.2 Hz) in **7**. Several 6-*O*-acylated grayanoids have been isolated from *Lyonia ovalifolia* D. *elliptica* Hand. -Mazz.,[7a] *Pieris japonica* D. Don,[7b] and *Rhododendron japonicum* Suringer.[7c] However, the new grayanotoxin is the first 6-*O*-acylate to be isolated from *Leucothoe grayana Max.* It causes skin inflammation upon contact.



	1	2	3	4	5	6	7
R_1	OH, H	OH, H	OH, H	OH, H	=O	=O	=O
R_2	$=CH_2$	$=CH_2$	CH$_3$, OH	CH$_3$, OH	CH$_3$, OH	CH$_3$, OH	$=CH_2$
R_3	OH, H	OAc, H	OH, H	OH, H	=O	=O	OAc, H
R_4	OH, H	OH, H	OH, H	OAc, H	OAc, H	OH, H	=O



8 Partial conformation of diketone **7**



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■ denotes a quaternary carbon atom

The second compound, G XVII ($C_{20}H_{30}O_6$, mp 268—270 °C (dec.), $[\alpha]_D$ −157.3 ° (*c* 1, MeOH)), was obtained in a 2.9×10^{-6}% yield from dried leaves and has the following spectral proterties: IR (cm^{-1}, Nujol) 3470 (OH), 1731 and 1712 (C=O); NMR (C_5D_5N, TMS) δ 1.02, 1.19, 1.42, 1.62 (each 3H, s, four tert. methyls), 2.92 (2H, bd, J=~11 Hz, C-2[8]), 3.90 (1H, q, J_{AX+BX}=9+12 Hz, C-1[8]), 2.84 (1H, d, J_{AB}=−13 Hz, C-7Hax), 4.22 (1H, d, J_{AB}=—13 Hz, C-7Heq), 5.27 (1H, s, C-14); ORD *a*=−463 (MeOH). The analytical and mass spectral data indicated that the compound corresponds to a bisdehydro derivative of G III (**3**). A comparison of the above spectral data with those of G III[1] suggested the presence of the C-14 hydroxyl group and two carbonyl groups as well as the absence of C-3 and C-6 protons. Moreover, the appreciable downfield shift of the C-14 proton of G XVII as compared to that of G III (δ 5.10, in C_5D_5N) also suggested the spatial closeness of the C-14 proton and a carbonyl group in G XVII (see **8**). Therefore, the 3,6-didehydro G III structure seemed suitable for G XVII.

This reasoning was proved correct by leading G I (**4**) with chromium trioxide in pyridine to 3,6-didehydro G I[6] (**5**) and by hydrolyzing the diketone with potassium carbonate in ethanol to give a product, which

was, in all respects, (IR, MS, NMR, and tlc) identical with G XVII. The new toxin was, therefore, formulated as 3,6-didehydro G III (**6**).

Two monoketo grayanoids, G V[2] and XIV,[3] were previously isolated and shown to possess no appreciable physiological activity. G XVII is the first grayanoid containing two carbonyl groups, and, in contrast to the monoketo grayanoids, it shows the sneeze-causing activity characteristic of polyhydroxylated grayanoids. The conformation of the central cycloheptane rings of G XVI and G XVII seems quite similar to that of G I on the basis of the NMR evidence presented above.

Experimental

All the mps are uncorrected. The NMR spectra were obtained on a Hitachi R-20B spectrometer, modified for INDOR experiments, in $CDCl_3$ or C_5D_5N, containing TMS as the internal reference. The chemical shifts are reported on the δ scale (s=singlet, q=quartet, d=doublet, bd=broad doublet). The IR spectra were measured on a JASCO Model IR-S spectrophotometer. The ORD curves were obtained on a JASCO ORD/UV-5 spectrometer. The specific rotations were measured in methanol at room temperature.

Isolation of G XVI and XVII. The powder (41 kg) of the leaves, which had been collected on Mt. Tarumae, Hokkaido, in July and dried, was extracted with hot water (*ca.* 4×10^3 l) for 3 days. The extracts were then concentrated under reduced pressure to *ca.* 70 l. An excess sat. lead acetate solution was added to the concentrate, and the ppt. was filtered off. An ammonium hydroxide solution was then added to the filtrate until the solution became alkaline (pH 8), after which the ppt. was filtered off again. H_2S gas was next bubbled through the filtrate to remove the Pb. After filtration and concentration, the solution was extracted with ethyl acetate. The organic phase was then dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a crude mixture consisting mainly of G I and III (368 g). After the removal of the solid material, the mother liquor was chromatographed on silica gel (2 kg, Wako, 100 mesh) and was roughly fractionated into A, B, and C. The first fraction, A (154 g), eluted with ethanol–chloroform (3:97, 2 l), was a mixture of several components and was rechromatographed on silica gel (600 g). A mixture containing G XVII (3 g, subfraction A_1) was first eluted with ethanol–chloroform (3:97, 0.8 l). Further elution with ethanol–chloroform gave, successively, G I, IV, V, and II (85, 13, 3, and 25 g respectively). The second fraction, B (252 g), a semicrystalline solid, was obtained by elution with ethanol–chloroform (5:95, 2 l). After the removal of the crystalline mixture of G I and II (125 g) with ethyl acetate, the filtrate was concentrated and the residue (126 g) was chromatographed on silica gel (800 g). Elution with ethanol–chloroform (3:97, 0.5 l) yielded a mixture (0.7 g, subfraction B_1) of leucothol A, C, and G XVII. Further elution with the same solvent afforded a mixture (5.8 g) of G I and XV, while elution with a more polar solvent system (7:93, 1.8 l) gave a mixture (119 g) of G I, II and V. Later fractions eluted with ethanol–chloroform (7:93, 3 l) contained, altogether, 282 g of G I, II, III, and V.

The above-described subfractions, A_1 and B_1, were then combined, and the mixture (3.7 g) was chromatographed on silica gel (400 g). Elution with ethanol–chloroform (2:98, 0.5 l) gave unknown compounds (268 mg). Further elution with the above solvent (3:97, 0.8 l) afforded leucothol C (53 mg) and then G XVII (118 mg): needles from methanol; mp 268—270 °C (dec.), $[\alpha]_D$ −157.3 ° (*c* 1, MeOH); ORD $[\phi]_{min}^{320nm}$ −20532 °C, $[\phi]_{max}^{275nm}$ +25752 °C; MS *m/e* 366 (M^+).

Found: C, 65.78; H, 8.27%. Calcd for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25%.

Further elution with the same solvent system (4:96, 0.8 l) gave a mixture of leucothol A and B (390 mg), while elution with the 5:95 solvent system (1.5 l) afforded, successively, crystals of GV (380 mg), XV (185 mg), and XVI (210 mg). The last compound was recrystallized from ether to give plates; mp 116—118 °C, $[\alpha]_D$ +2.8 ° (*c* 1, MeOH); MS *m/e* 394 (M^+).

Found: C, 66.88; H, 8.69%. Calcd for $C_{22}H_{34}O_6$: C, 66.78; H, 8.44%.

Hydrolysis of G XVI. G XVI (37 mg) was dissolved in a mixture of aqueous sodium hydroxide (5%, 0.5 ml) and ethanol (2 ml), and the solution was heated under reflux for 1 hr. After cooling, the solution was diluted with excess ethyl acetate, washed with water and brine, and dried over anhydrous sodium sulfate. The evaporation of the solvent gave crystals (34 mg) identical (IR, NMR, and tlc) with those of G II.

Oxidation of G XVI. To a chromium trioxide–pyridine complex solution prepared from 90 mg of chromium trioxide and 1 ml of pyridine, a solution of G XVI (30 mg) in pyridine (2 ml) was added, after which the whole solution was left to stand for 1 day at room temperature. An usual work-up gave an amorphous solid (26 mg) which was purified from ethyl acetate. IR (cm^{-1}, film) 3480, 1745 (C=O), 1720 (O_2CCH_3), 1630 (C=C); NMR ($CDCl_3$) 1.08, 1.11, 1.42 (each 3H, s, C–CH_3), 2.06 (3H, s, O_2CCH_3), 5.00, 5.19 (each 1H, bs, C=CH_2), 6.39 (1H, q, J_{AX+BX}=4+9 Hz, C-6); MS *m/e* 390 (M^+).

Hydrolysis of 3,6-Didehydro G I (5).[6] A mixture of **5** (111 mg) and potassium carbonate (17 mg) in ethanol (5 ml) was stirred for 20 min at room temperature. After the evaporation of ethanol under reduced pressure, the residue was dissolved in ethyl acetate and the solution was washed with water and brine and dried over anhydrous sodium sulfate. The subsequent removal of the solvent gave crude crystals which were purified with ethyl acetate to afford prisms (85 mg). The crystalline product was identical with G XVII in all respects (IR, NMR, MS, and tlc).

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8) Tentative assignment. It appears that in general, C-1 and C-2 protons of Gs resonate at unusually low field in pyridine and exhibit unusually large coupling constants S). Gasa and T. Matsumoto, unpublished observations).