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# GRAYANOSIDE C, A NEW DITERPENE GLUCOSIDE FROM *LEUCOTHOE GRAYANA*

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**Key Word Index**—*Leucothoe grayana*; Ericaceae; diterpene glucoside; grayanoside C; leucothol A; X-ray structure determination.

**Abstract**—A new diterpene glucoside has been isolated from *Leucothoe grayana*. Its structure was elucidated by X-ray diffraction analysis of a dehydrated product of the aglycone and conversion of the same to leucothol A.

## INTRODUCTION

In previous papers [1, 2] we reported the isolation of two grayanoid glucosides, grayanoside A and B, from *Leucothoe grayana*. This paper describes the isolation and structure determination of another new grayanoid glucoside, grayanoside C, from the same plant.

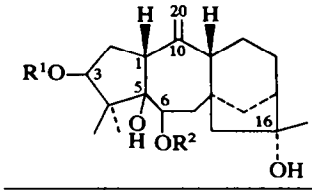
## RESULTS AND DISCUSSION

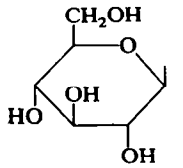
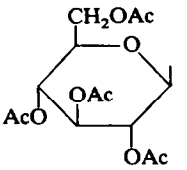
Grayanoside C (**1a**), viscous syrup, was isolated from the *n*-BuOH-soluble fraction of an MeOH extract as well as grayanoside A and B. Acetylation of **1a** gave a pentaacetate (**1b**), $C_{36}H_{52}O_{14}$, mp 220–222°.

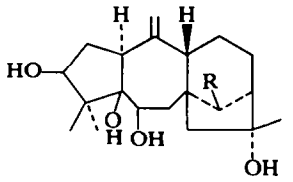
Acid hydrolysis of **1a** yielded glucose, but its aglycone could not be obtained. Enzymatic hydrolysis of **1a** with naringinase gave a genuine aglycone (**2a**), $C_{20}H_{32}O_4$, mp 232–233° (decomp.). Acetylation of **2a** yielded a diacetate (**2b**), syrup. These findings and comparison of the spectral data (see Experimental) suggested that **2a** has the same composition and functional groups as grayanotoxin-XVIII (**3**) [2]: three tertiary methyls, six methylenes, three methines, two quaternary carbons, two secondary hydroxyls, two tertiary hydroxyls and a vinylidene.

To confirm the structure, we tried to undertake an X-ray diffraction analysis of the aglycone (**2a**), but a suitable crystal could not be obtained. Then **2a** was refluxed with anhydrous $CuSO_4$ in dioxane to yield a monodehydrated product (**4**), $C_{20}H_{30}O_3$, mp 205–206° (decomp.), which was submitted for X-ray analysis. The stereoscopic drawings of the molecules, which revealed the chemical structure of **4**, 3β,5β,6β-trihydroxy-(1βH)-grayana-10(20),15-diene are shown in Fig. 1. Hence the structure of the original aglycone (**2a**) was assumed to be 3β,5β,6β,16ξ-tetrahydroxy-(1βH)-grayan-10(20)-ene. At this stage, however, the configuration of 16-hydroxyl group, and the glucosidation pattern and position in the glucoside (**1a**) remained uncertain.

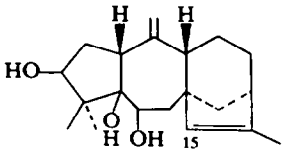
The aglycone (**2a**) was treated with methanesulfonyl chloride to give a monodehydrated product (**5**), $C_{20}H_{30}O_3$, mp 245°, other than **4**. The IR spectrum of **5** showed a carbonyl absorption (1702 $cm^{-1}$) and the $^1H$ NMR revealed the presence of three tertiary methyls, one secondary hydroxyl group and one exo-methylene group. Hikino and his coworkers reported that grayanotoxin-II (**6**) reacted with *p*-toluenesulfonyl chloride to yield a rearranged product (**7**), whose CD curve exhibited a positive Cotton effect [4]. Since the CD curve of **5** showed a negative Cotton effect and the starting material (**2a**) had 1β-configuration, **5** must have a rearranged six–six-membered A–B ring and 1β,6α-configuration [4].



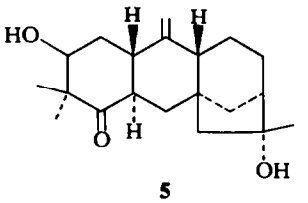
| | $R^1$ | $R^2$ |
|---|---|---|
| **1a** |  | H |
| **1b** |  | Ac |
| **2a** | H | H |
| **2b** | Ac | Ac |



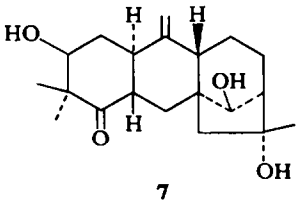
3 R = H
6 R = OH



4



5



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From these findings, the structure of **5** was deduced to be leucothol A, which was previously isolated from *Leucothoe grayana* [5], and was identified. Consequently the 16-hydroxyl group of the aglycone (**2a**) should have an $\alpha$-configuration like **5**.

In the $^1$H NMR spectra the anomeric proton of **1a** and **1b** was observed at $\delta$ 4.88 (*d*, $J$ = 7 Hz) and 4.58 (*d*, $J$ = 7 Hz), respectively, so **1a** must be a $\beta$-D-glucoside. The glucosidation position was determined by the $^{13}$C NMR spectra. The $^{13}$C NMR signals were assigned by means of single-frequency off-resonance decoupling, selective proton decoupling and by comparing the spectra of several analogous compounds. In general, carbinyl carbon ($\alpha$-carbon) signals of aglycone alcohols are displaced by 5.5-10 ppm on glucosidation [6, 7]. The C-3 signal of the aglycone (**2a**) appeared at $\delta$ 83.6, while that of the glucoside (**1a**) was observed at $\delta$ 92.0. Other carbinyl carbon signals scarcely shifted on glucosidation. Hence $\beta$-D-glucose must be bound at C-3 of the aglycone (**2a**).

From the above data, grayanoside C (**1a**) was concluded to be 3-*O*-($\beta$-D-glucopyranosyl)-(1$\beta$H)-grayanotoxin-XVIII. This is the first grayanoid glucoside, epimerized at C-1, to be found in nature. In the course of our investigation, Furusaki and his coworkers reported the isolation of grayathol A from the same plant as ours, whose structure was identical with **4** [8].

## EXPERIMENTAL

Mps were uncorr. $^1$H NMR spectra were measured at 100 MHz. $^{13}$C NMR spectra were measured at 15 MHz. The $\delta$ values are expressed in ppm downfield from TMS int. standard. MS (20 eV) were taken with a direct inlet.

*Isolation of* **1a**. **1a** was obtained in the course of isolation of grayanoside A and B [1, 2]. Activated charcoal column chromatography of the *n*-BuOH extract afforded crude **1a** in the fractions eluted with MeOH–$H_2O$ (8:2 to 9:1), 5 g of which was chromatographed on a Si gel column. The $CHCl_3$–MeOH (9:1) eluate gave **1a** (2.2 g), which was purified by silanised Si gel PLC (developing solvent: MeOH–$H_2O$ (1:1)).

*Grayanoside C* (**1a**). Viscous syrup. $[\alpha]_D^{24}$ +6.00° (MeOH *c* 2.00). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3390, 1075. $^1$H NMR ($C_5D_5N$): $\delta$ 1.17, 1.50, 1.70 (each 3H, *s*), 3.8–4.5 (many protons), 4.88 (1H, *d*, $J$ = 7 Hz), 5.26 (*s*). $^{13}$C NMR ($C_5D_5N$): $\delta$ 21.1 (Me), 23.4 (Me), 24.8 (Me), 26.7 (C-11 and C-12), 35.2 (C-2), 35.6 (C-14), 45.7 (C-8), 48.8 (C-7), 49.6 (—CH—), 51.5 (C-4), 55.3 (—CH—), 57.3 (C-15), 59.0 (—CH—), 62.7 (glucose C-6), 71.2 (C-6), 71.6 (glucose C-4), 75.6 (glucose C-2), 78.6 (C-16 and glucose C-3, C-5), 86.1 (C-5), 92.0 (C-3), 105.0 (glucose C-1), 110.3 (—C=$CH_2$), 153.9 (—C=$CH_2$).

*Pentaacetylgrayanoside C* (**1b**). Treatment of **1a** with $Ac_2O$–Py overnight at room temp. gave **1b**, mp 220–222° (MeOH). (Found: C, 60.55: H, 7.45. Calc. for $C_{36}H_{52}O_{14}$: C, 61.00; H, 7.39%). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3545, 1755, 1230. $^1$H NMR ($CDCl_3$): $\delta$ 0.81, 1.07, 1.36 (each 3H, *s*), 2.03 (3H×3, *s*), 2.07 (3H×2, *s*), 4.58 (1H, *d*, $J$ = 7 Hz).

*Acid hydrolysis of* **1a**. A soln of **1a** (4 mg) in dioxane (1 ml) and 5% $H_2SO_4$ (2 ml) was heated for 2.5 hr on a steam bath. The mixture was cooled, diluted with $H_2O$ (10 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

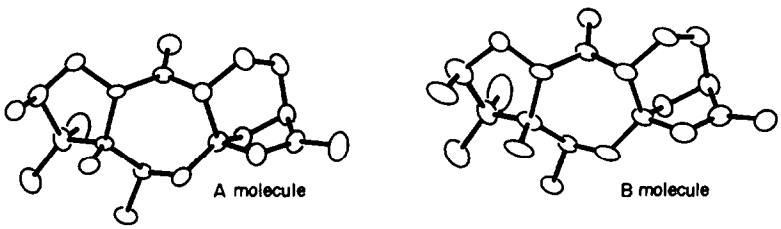


Fig. 1. Stereoscopic views of the structures of molecles A and B of **4**. The figures were drawn by an ORTEP program by Johnson [3].

derivative and identification was made by comparison of $R_t$ of the authentic TMSi-D-glucose by GLC. GLC was performed at 170° using a stainless column (2m × 3mm) of 5% OV-1 on Chromosorb W(AW).

*Enzymatic hydrolysis of* **1a**. To a soln of **1a** (100 mg) dissolved in HOAc–NaOAc buffer (pH 4.1, 10 ml) was added crude naringinase 'Sankyo' (100 mg) and incubated at 37°. After 24 hr, 50 mg of naringinase was added and further incubated for 24 hr. The soln was extracted with EtOAc and purified by Si gel PLC (eluent: $CHCl_3$–MeOH, 9:1) to give the aglycone (**2a**, 39 mg). Mp 232–233° (decomp.) (EtOAc). (Found: C, 71.37; H, 9.73. Calc. for $C_{20}H_{32}O_4$; C, 71.39; H, 9.59%). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3370, 1635. $^1H$ NMR ($CDCl_3$): $\delta$ 0.98, 1.24, 1.38 (each 3H, *s*), 3.58–3.78 (2H, *m*), 5.18 (2H, *br s*). $^{13}C$ NMR ($C_5D_5N$): $\delta$ 21.1 (Me), 22.8 (Me), 24.8 (Me), 26.6 (C-11 or C-12), 26.8 (C-12 or C-11), 35.6 (C-14), 36.8 (C-2), 45.7 (C-8), 48.8 (C-7), 49.6 (—CH—), 50.8 (C-4), 55.7 (—CH—), 57.3 (C-15), 58.7 (—CH—), 71.1 (C-6), 78.6 (C-16), 83.6 (C-3), 86.8 (C-5), 110.5 (—C=$CH_2$), 153.9 (—C=$CH_2$).

*Acetylation of* **2a**. Treatment of **2a** (11 mg) with $Ac_2O$–Py overnight at room temp. gave a diacetate (**2b**) (12 mg), which was purified by Si gel PLC (eluent: $CHCl_3$–MeOH, 19:1). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3490, 1730, 1632, 1245. $^1H$ NMR ($CDCl_3$): $\delta$ 0.92 1.08, 1.37 (each 3H, *s*), 2.06, 2.11 (each 3H, *s*), 4.84 (1H, *d*, $J$ = 10 Hz), 5.02 (1H, *dd*, $J$ = 6 and 10 Hz), 5.19 (1H, *br s*), 5.27 (1H, *br s*). MS *m/e:* 360 ($M^+$ – HOAc), 342 ($M^+$ – HOAc – $H_2O$), 300 ($M^+$ – 2HOAc), 282 ($M^+$ – 2HOAc – $H_2O$).

*Dehydration of* **2a**. To a soln of **2a** (52 mg) in dioxane (5 ml) was added dry $CuSO_4$ (50 mg) and refluxed for 8 hr. After filtration of $CuSO_4$, the filtrate was evapd *in vacuo* and purified by Si gel PLC (eluent: $CHCl_3$–MeOH, 9:1) to yield **4** (34 mg). Mp 205–206° (decomp.) (EtOAc). (Found: C, 75.60; H, 9.68. Calc. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50%). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3325, 1630. $^1H$ NMR ($C_5D_5N$): $\delta$ 1.12, 1.57 (each 3H, *s*), 1.62 (1H, *d*, $J$ = 2 Hz), 3.00 (2H, *m*), 3.80 (1H, *m*), 3.93 (1H, *m*), 5.07 (1H, *d*, $J$ = 2 Hz), 5.13, 5.23 (each 1H, *s*). MS *m/e* 318 ($M^+$), 300 ($M^+ - H_2O$), 282 ($M^+ - 2H_2O$).

*X-ray analysis.* Crystals grown from EtOAc solns were colourless prisms elongated along the *c*-axis. The lattice constants and intensity data were collected on a Philips PW 1100 diffractometer using CuK$\alpha$ radiation monochromated by a graphite plate.

*Crystal data.* $C_{20}H_{30}O_3$, MW = 318.5, orthorhombic, space group $P2_12_12_1$, $Z$ = 8, $D_x$ = 1.189 g/cm$^3$, $a$ = 32.137 (15), $b$ = 15.150 (7), $c$ = 7.308 (4) Å, $U$ = 3558.1 Å$^3$. The unit cell contains two crystallographically independent molecules A and B. Intensities of 3832 independent structure factors were obtained as above the $2\sigma$(I) level within the $2\theta$ angle of 156°. The crystal structure was solved by the direct method using MULTAN [9] and refined by the block-diagonal least-squares calculations using HBLS [10]. The atomic coordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K.

*Reaction of* **2a** *with methanesulfonyl chloride.* **2a** (30 mg) was dissolved in Py (1 ml) and a small amount of MsCl (3 drops) was added. After 15 min at room temp., $H_2O$ was added and extracted with EtOAc. The EtOAc layer was washed with $H_2O$, 5% KOH soln and $H_2O$, successively. After removal of the solvent, the residue was purified by Si gel PLC (eluent: $CHCl_3$–MeOH, 9:1) to yield 13 mg of colourless crystals (**5**). Mp 245° (EtOAc). (Found: C, 75.35; H, 9.76. Calc. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50%). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3495, 3385, 1702, 1640. $^1H$ NMR ($C_5D_5N$): $\delta$ 1.16, 1.46, 1.54 (each 3H, *s*), 4.18 (1H, *t*, $J$ = 3 Hz), 4.89, 5.08 (each 1H, *d*, $J$ = 1 Hz). MS *m/e* 318 ($M^+$), 300 ($M^+ - H_2O$), 282 ($M^+ - 2H_2O$). CD $[\theta]_{290.5}$ – 12 500 (MeOH, $c = 4.6 \times 10^{-4}$).

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