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NEW A-NOR-B-HOMO-(−)-KAURANOIDS FROM *LEUCOTHOE GRAYANA MAX*

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Abstract—Two physiologically active diterpenoids, grayanotoxin IV and V, were isolated from *Leucothoe grayana Max*. The structures including the absolute configurations **4** and **5** respectively are supplied by spectral and chemical evidence.

STRUCTURES of a number of diterpenoids with the A-nor-B-homo-(−)-kaurane skeleton have been elucidated.[1a–c] In this paper we report the isolation from *L. grayana Max.* of two physiologically active* diterpenoids of this type, for which we suggest the names grayanotoxin IV and V.† These compounds were obtained by careful chromatography of the toxic fraction of the alcohol extracts of leaves together with grayanotoxins I (**1**) and III (**3**). Grayanotoxins IV and V are not artefacts formed during isolation, since TLC of fresh ethanolic or acetone-water extracts indicate the presence of these compounds in the leaves.

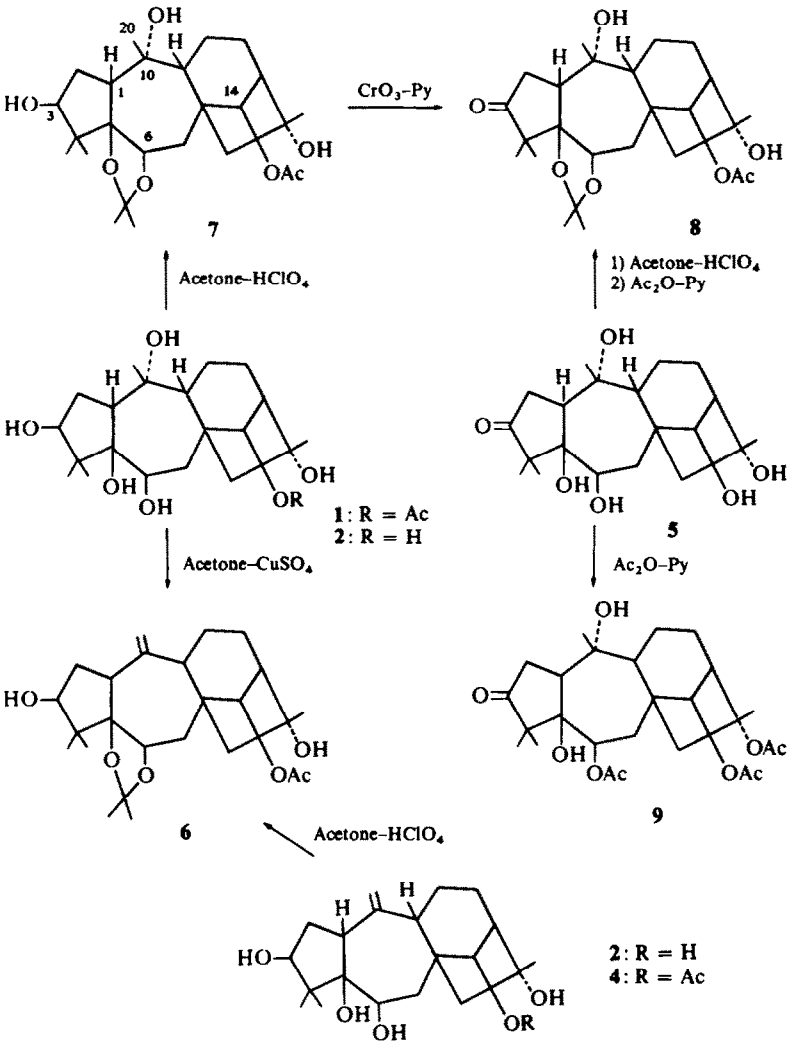
The first compound, grayanotoxin IV, $C_{22}H_{34}O_6$, m.p. 174–175°, $[\alpha]_D$ − 18·6° (*c*, 1·0, MeOH) was obtained in 0·09% yield from dried leaves. IR and NMR spectra indicate presence of the following groups: two tertiary Me's (δ 0·93 and 1·15), a tertiary Me on a carbon bearing oxygen (δ 1·27), hydroxyls (3480 and 3280 cm^{-1}) a secondary acetoxyl (1711 cm^{-1}, δ 2·10 and 5·28, s) and an exocyclic methylene group (1630 cm^{-1}, δ 4·96 and 5·08, each s). Comparison of these data with those of grayanotoxin I (**1**) (which exhibits a singlet NMR line for C-14 proton), and grayanotoxin II (**2**) (C=CH_2, 1630 cm^{-1}) suggested that the new toxin may be 14-O-acetylgrayanotoxin II.

Although this compound has been described,[2] reported chemical shifts were significantly different from those of grayanotoxin IV. However, the above reasoning was proved to be correct. On treatment with acetone and a catalytic amount of perchloric acid, grayanotoxin IV gave an acetonide (**6**), m.p. 212·5–214·5°, $[\alpha]_D$ − 20° (*c*, 1·0, MeOH), ν_{max} (nujol) 1628 cm^{-1} (C=CH_2), δ ($CDCl_3$) 5·00 (3H, b. s, C=CH_2 and C—14H), identical with the monoanhydro acetonide derived from grayanotoxin I.[1a, 2a] The position of an exocyclic double bond in **6** was deduced to be on the 7-membered ring by IR frequency (*ca*, 1630 cm^{-1}) characteristic for grayanotoxin II (**2**) derivatives‡ and by upfield shift of NMR signal due to C-14 proton upon changing **1**

* See Experimental

† This substance has been quite recently isolated from the same plant by T. Takemoto and H. Hikino (Tohoku University, Sendai, Japan, private communication).

‡ By contrast an exocyclic double bond at C-16 position on the 5-membered ring of A-nor-B-homo-kaurane skeleton absorbs at 1650 cm^{-1}.[1a]



to **6**.* The grayanotoxin IV is therefore formulated as the 14-O-acetyl derivative of grayanotoxin II (**2**) and expressed by the formula **4**.

The second compound, grayanotoxin V, $C_{20}H_{32}O_6$, m.p. 230–232° (dec), $[\alpha]_D$ −61·5° (*c*, 1·0, MeOH) was obtained in 0·003% yield from the dried leaves and has the following spectral properties; ν_{max} (nujol) 3390 (OH), 1725 cm^{-1} (C=O), δ (pyridine) 1·17, 1·45, 1·53 and 1·71 (each 3H, s). The compound was acetylated to give a triacetate (**9**), m.p. 184–185°, which still contains at least one OH group (ν_{max} (nujol) 3380 cm^{-1}) and exhibits NMR signals at δ ($CDCl_3$) 0·96, 1·05, 1·38, 1·59 (each 3H, s), 1·95, 2·03, 2·12 (each $OCOCH_3$), 4·95 (1H, q) and 5·46 (1H, s). Therefore the compound contains

* Such upfield shift has been observed invariably on dehydration at C-10 of grayanotoxin I (**1**) derivatives.[1a]

four tertiary Me's, a CO group, two secondary and at least two tertiary OH groups, of which one is acylable.* The singlet peak due to a methine bearing acetoxyl group at such a low field as δ 5·46 is characteristic of the C-14 proton in grayanotoxin I (**1**) and together with the above spectroscopic data suggests that grayanotoxin V is a monodehydrograyanotoxin III. Either the C-3 or C-6 OH group of the latter compound is replaced by an oxo group in grayanotoxin V. The CO group is probably located at C-3 position, since the ORD curve (a = −72, MeOH) of grayanotoxin V is superposable on that of other 3-dehydrograyanotoxin derivatives.[1] In order to determine the position of the CO group, grayanotoxin V was treated with acetone in the presence of perchloric acid to give an acetonide, which was then acetylated to furnish a monoacetate (**8**), m.p. 190–191°, ν_{max} (nujol) 1745 and 3560 cm^{-1} (C=O and OH), δ ($CDCl_3$) 5·88 (1H, s, C-14H). On the other hand, grayanotoxin I (**1**) yielded an acetonide (**7**) monohydrate, m.p. 142–144°, on treatment with acetone-perchloric acid and subsequent oxidation of the acetonide (**7**) with CrO_3-pyridine gave rise to a ketone, which was in all respects identical with the above acetate (**8**). Therefore grayanotoxin V is a 3-dehydro compound of grayanotoxin III (**3**) and expressed by the formula **5**.

EXPERIMENTAL

All m.ps are uncorrected. The NMR spectra were obtained on Hitachi H-60 spectrometer in $CDCl_3$ containing TMS as internal reference, unless otherwise stated. Chemical shifts are reported as parts per million on the δ scale (s = singlet, q = quartet, b.q = broad quartet and m = multiplet). The IR spectra were measured on a JASCO Model IR-S spectrophotometer. The ORD curves were obtained on a JASCO ORD/UV-5 spectrometer. Specific rotations were measured in $CHCl_3$ at room temp.

Isolation of grayanotoxin IV (**4**) *and grayanotoxin* V (**5**)

The powder (1·3 kg) of the dried leaves, which were collected on Mt Tarumae, Hokkaido in July, was extracted with EtOH (12 l) for 3 days at room temp. This operation was repeated 3 times. The combined alcohol soln was evaporated under reduced press and water was added to the concentrate. Excess saturated lead acetate was added to the solution and the ppt was filtered off. Then, NH_4OH was added to the filtrate until the soln became alkaline (pH = 8) and the ppt was again filtered off. HS was then bubbled through the filtrate to remove Pb. The soln was filtered and the clear soln was concentrated under reduced press to give an oily residue. The residue was extracted with EtOAc (200 ml) 3 times and the combined EtOHAc soln was evaporated under reduced press to give a crude crystalline material. Recrystallization from EtOAc gave crystals (13·4 g) of a mixture of **1** and **3**.

The mother liquor was chromatographed on silica gel (110 g, Merck, less than 0·08 mm) and eluted with EtOH-$CHCl_3$. Elution with EtOAc-$CHCl_3$ (3:97) gave 1·12 g of **4**. Elution with EtOH-$CHCl_3$ (5:95) gave 2 g of **1** and further elution with the same solvent gave crystals (39 mg) of **5**. Finally elution with EtOH-$CHCl_3$ (1:9) gave 1 g of **3**. Recrystallization of **4** from EtOAc gave crystals, m.p. 174–175°, $[\alpha]_D$ − 18·6° (*c*, 1·0, MeOH); IR ν_{max}^{nujol} 3480 (OH), 1711 (OAc) and 1630 (C=CH_2) cm^{-1}; NMR δ 0·93, 1·15 and 1·27 (each 3H, s), 2·10 (3H, s), 5·08 and 4·96 (each 1H, s) and 5·28 (1H, s). (Found: C, 67·01; H, 8·70, $C_{22}H_{34}O_6$ requires: C, 66·98; H, 8·96%). Recrystallization of **5** from EtOAc gave crystals, m.p. 230–232° (dec), $[\alpha]_D$ − 61·5° (*c*, 1·0, MeOH); IR ν_{max}^{nujol} 3390 (OH) and 1725 (C=O) cm^{-1}; NMR δ (pyridine) 1·17, 1·45, 1·53 and 1·71 (each 3H, s). (Found: C, 65·20; H, 8·82. $C_{20}H_{32}O_6$ requires: C, 65·19; H, 8·75%).

Grayanotoxins IV and V were also obtained by extraction of the dried leaves with acetone-water instead of EtOH. Grayanotoxins IV and V are both physiologically active. Grayanotoxin IV causes sneezing and irritates the tongue like grayanotoxins I, II and III. Grayanotoxin V irritates the tongue likewise, but does not show the sneeze-causing activity.†

* For similar examples of acetylation of a C-16 tertiary OH group in grayanotoxins see a paper by T. Matsumoto and M. Watanabe in Ref 1*a*.

† The Japanese name for *Leucothoe grayana*, hanahirinoki, means sneeze-causing shrub. Detailed studies on the physiological activity of the new toxins will be reported elsewhere.

Reaction with acetone of grayanotoxin I *in the presence of anhydrous cupric sulfate*

To a soln of 5 g of **1** dissolved in 1 l dry acetone, 140 g anhyd $CuSO_4$ (baked at 300° in air bath) was added and refluxed with stirring for 18 hr. After cooling the $CuSO_4$ was filtered off and the acetone soln was concentrated under reduced press to give an oily material. The product was chromatographed on 50 g silica gel and elution with benzene-EtOAc (1:1) gave 2 g dianhydro acetonide[1a, 2a] and 250 mg of **6**, m.p. 223° (from cyclohexane), $[\alpha]_D$ − 20° (*c*, 1·0, MeOH); IR $\nu_{max}^{CHCl_3}$ 3555 (OH), 1744 and 1244 (OAc) and 1629 ($C=CH_2$) cm^{-1}; NMR δ 0·84, 1·06 (each 3H, s), 1·34 (9H, s), 2·07 (3H, s), 3·64 (1H, m), 4·20 (1H, b.q), 5·00 (3H, m).

Reaction with acetone of grayanotoxin IV (**4**) *in the presence of perchloric acid*

To a soln of 150 mg of **4** dissolved in 30 ml dry acetone, 0·02 ml 60% perchloric acid was added and the whole was kept for 30 min at room temp. To the reaction mixture saturated $NaHCO_3$ was added and the resultant soln was concentrated under reduced press. The residue was extracted with EtOAc, and the soln was washed with water, dried over Na_2SO_4 and evaporated under reduced press. The oily material was crystallized from a small amount of EtOAc and gave 132 mg of crystals, which were identical in all respects with **6**.

Grayanotoxin I-5,6-*acetonide* **7**

To a soln of 1 g of **1** in 130 ml dry acetone, 0·2 ml 60% perchloric acid was added and kept for 30 min at room temp. Saturated $NaHCO_3$ was added to the rn mixture and the resultant soln was concentrated under reduced press. The residue was extracted with benzene 3 times, the combined benzene soln was washed with water and dried over Na_2SO_4. The solvent was then evaporated under reduced press. The residue was chromatographed on silica gel and elution with benzene-EtOAc (1:1) gave 630 mg of **7** as a monohydrate, m.p. 142·5–144° (from EtOAc), $[\alpha]_D$ − 6° (*c*, 1·0, MeOH); IR ν_{max}^{nujol} 3420 and 3320 (OH), 1710 and 1260 (OAc) and 1650 (H_2O); NMR δ 0·88, 1·12, 1·55 and 1·62 (each 3H, s), 1·38 (6H, s), 2·12 (3H, s, OAc), 2·45 (H_2O and OH, disappeared by treatment with D_2O), 3·63 (1H, m), 4·24 (1H, q) and 5·86 (1H, s). (Found: C, 64·09; H, 8·94. $C_{25}H_{40}O_7 \cdot H_2O$ requires: C, 63·80; H, 9·00%).

3-*Dehydrograyanotoxin* I-5,6-*acetonide* (**8**)

A. To a complex of pyridine (2 ml) and CrO_3 (200 mg), 100 mg of **7** dissolved in 1 ml pyridine was added and allowed to stand overnight. The mixture was filtered and the filtrate was diluted with EtOAc, washed with water, dried over $NaSO_4$ and evaporated under reduced press. The residue was crystallized from ether to give 47 mg of **8*** as a monohydrate, m.p. 190–191°, $[\alpha]_D$ − 52° (*c*, 1·0, MeOH); IR ν_{max}^{nujol} 3580 and 3410 (OH), 1745, 1735 (shoulder), 1238 (C=O and OAc) and 1650 (H_2O) cm^{-1}; NMR δ 0·94, 1·03, 1·30, 1·33, 1·40 and 1·47 (each 3H, s), 2·08 (3H, s, OAc), 4·27 (1H, q), 5·88 (1H, s). (Found: C, 64·17; H, 8·83. $C_{25}H_{38}O_7 \cdot H_2O$ requires: C, 64·08; H, 8·60%).

B. To a soln of 40 mg **5** dissolved in 5 ml acetone, 0·01 ml 60% perchloric acid was added and kept for 15 min at room temp. Saturated $NaHCO_3$ was added to the mixture and acetone was removed under reduced press at low temp. The residue was extracted with EtOAc 3 times and the combined EtOAc soln was washed with water, dried over Na_2SO_4 and evaporated under reduced press. The residue was used without purification. To a soln of the residue dissolved in 0·5 ml pyridine, 0·5 ml Ac_2O was added and the whole was allowed to stand overnight at room temp. The mixture was poured onto a crushed ice and extracted with EtOAc. The combined organic layer was washed with saturated $NaHCO_3$ and water, dried over $NaSO_4$ and evaporated under reduced press. The residue was purified by preparative TLC on silica gel (Wako, B-5) and gave 10 mg of crystals, m.p. 190·5° (from ether), which were identical in all respects with **8** derived from **1**.

Triacetyl grayanotoxin V (**9**)

To a soln of 50 mg of **5** dissolved in 1 ml pyridine, 1 ml Ac_2O was added and the soln was refluxed for 30 hr. The mixture poured into ice water and the resultant soln was extracted 3 times with ether. The combined ether layers were washed with water, dried over Na_2SO_4, and evaporated *in vacuo* to give crystals. Recrystallization from ether gave **9**, m.p. 184–185°, ν_{max} (nujol) 3380 (OH), 1740, 1710 (C=O, $OCOCH_3$) cm^{-1}, NMR δ 0·96, 1·05, 1·38, 1·59 (each 3H, s), 1·95, 2·03, 2·12 (each 3H, $OCOCH_3$), 4·95 (1H, q, J = 5·9 Hz), and 5·46 (1H, s). (Found: C, 62·94; H, 7·61, $C_{26}H_{38}O_9$ requires: C, 63·14; H, 7·75%).

* Tallent[2a] prepared this ketone by CrO_3-H_2SO_4 oxidation (m.p. 200–202° from EtOAc-cyclohexane).

REFERENCES

[1] [a] Grayanotoxin I, II and III, T. Matsumoto and M. Watanabe, *Tetrahedron Letters* 6019 (1968); H. Kakisawa, T. Kojima, M. Yanai and K. Nakanishi, *Tetrahedron* **21**, 3091 (1965) and refs cited;
[b] Rhodojaponin I, II, III and Asebotoxin III, H. Hikino, K. Ito, T. Ohta and T. Takemoto, *Chem. Pharm. Bull.* **17**, 1078 (1969);
[c] Asebotoxin I and II, H. Hikino, K. Ito and T. Takemoto, *Ibid.* **17**, 854 (1969)

[2] [a] W. H. Tallent, *J. Org. Chem.* **29**, 2756 (1964);
[b] H. Meguri, *J. Pharm. Soc. Japan* **79**, 1060 (1959)