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STEREOSTRUCTURE OF GRAYANOL A AND B, DITERPENOIDS OF *LEUCOTHOE GRAYANA*

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From the leaves of *Leucothoe grayana* Maximowicz (Ericaceae), a famous poisonous shrub in Japan, fifteen toxic diterpenoids of the andromedane skeleton, grayanotoxin I - XV, and four diterpenoids of the leucothane skeleton, leucothol A, B, C, and D, have been isolated.[1-5] Continuation of our investigation has led to the isolation of two new diterpenoids possessing the novel carbon skeleton for which the names grayanol A and B (G-A and G-B) are proposed.

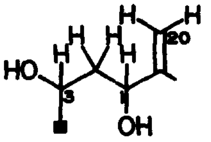
G-A, $C_{20}H_{32}O_6$ (MS *m/e* 368 (M^+)), m.p. 174-175°, is indicated by the IR and ^1H NMR spectra† to contain three tertiary methyls (δ 1.40, 1.54, 1.65 ppm), a vinylidene (ν_{max} 1640, 893 cm^{-1}, δ 5.45, 5.62 ppm), a saturated carbonyl in a six- or larger-membered ring (ν_{max} 1701 cm^{-1}), and hydroxyls (ν_{max} 3400, 3200 cm^{-1}) in which four are secondary (δ 4.30, 4.56, 4.69, 5.00 ppm). The ^{13}C NMR spectrum classifies the twenty carbons into three methyls (δ 16.4, 24.0, 25.9 ppm), five methylenes (δ 26.6, 27.2, 35.4, 45.3, 57.8 ppm), two methines (δ 51.7, 53.9 ppm), two quaternary carbons (δ 53.3, 54.3 ppm), four tertiary carbinyl carbons (δ 67.9, 70.7, 79.2, 85.7 ppm), one quaternary carbinyl carbon (δ 80.4 ppm), one vinylidene (δ 115.2, 150.9 ppm), and a carbonyl (δ 215.6 ppm).

G-B, $C_{20}H_{32}O_6$ (MS *m/e* 368 (M^+)), m.p. 195-196°, possesses the spectral properties showing that G-B contains the same functional groups as G-A: *i.e.*, three tertiary methyls (δ 1.42, 1.44, 1.52 ppm), a vinylidene (ν_{max} 1640, 910 cm^{-1}, δ 5.54, 5.78 ppm), a saturated carbonyl in a six- or larger-membered ring (ν_{max} 1700 cm^{-1}), and hydroxyls (ν_{max} 3560, 3370 cm^{-1}) in which four are secondary (δ 4.30, 4.72, 4.85, 5.25 ppm). Further, the ^{13}C NMR spectrum also indicates the presence of three methyls (δ 16.2, 23.5, 25.6 ppm), five methylenes (δ 25.6, 27.9, 38.4, 45.5, 53.5 ppm), two methines (δ 50.7, 53.5 ppm), two quaternary carbons (δ 53.9, 58.0 ppm), four tertiary carbinyl carbons (δ 67.4, 69.8, 73.1, 78.9 ppm), one quaternary carbinyl carbon (δ 79.9 ppm), one vinylidene (δ 111.6, 157.1 ppm), and a carbonyl (δ 207.7 ppm) as in G-A.

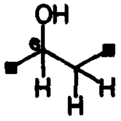
The mass spectra of G-A and G-B bear the close resemblance though minor differences in the relative intensities in certain peaks are detectable, and alkali treatment of G-A led to the conversion into G-B. These facts indicate that both substances are closely related each other and, most likely, stereoisomers.

Analysis of the ^1H NMR spectrum of G-B with the aid of double resonance experiments demonstrated the presence of the partial structures A, B_1, and C_1. The situation of the (C-6) secondary hydroxyl adjacent to the carbonyl is suggested by the observations that, in the ^1H NMR spectrum, the carbinyl hydrogen is considerably deshielded (δ 5.25 ppm) and that G-B consumed the reagent on periodate oxidation. G-B was reduced with sodium borohydride to afford the dihydro-G-B (III), m.p. 144-146°, which on acetylation with acetic anhydride in pyridine gave the triacetate (IV). In the ^1H NMR spectrum of the triacetate (IV), the chemical shift (δ 4.98 ppm) of the (C-5) carbinyl hydrogen geminal to the newly formed hydroxyl demonstrates the hydroxyl to be free and its splitting pattern as a doublet originating from the coupling only with the (C-6) carbinyl hydrogen at 5.89 ppm indicates the location of the newly formed (C-5) carbinyl group to be adjacent to the (C-6) carbinyl group and also to be next to a quaternary carbon. The partial formula B_1 is thus extended to B_2. Further, an intramolecular nuclear Overhauser effect was observed between one of the (C-20) vinylidene hydrogens at 5.54 ppm and the (C-14) carbinyl hydrogen at 4.85 ppm, showing the spatially close location of the two hydrogens.

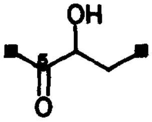
■ shows a quaternary carbon



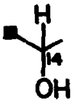
A



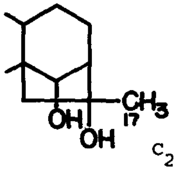
B_1



B_2



C_1



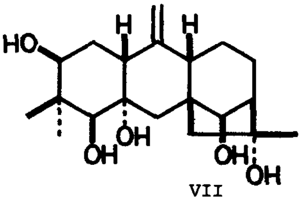
C_2

Acetylation of G-B with acetic anhydride in pyridine yielded the triacetate (V), m.p. 114-116°. The ^1H NMR spectrum of the triacetate (V) demonstrates that the three (C-1, C-3, and C-6) hydroxyls have been acetylated (δ 2.05, 2.11, 2.13, 4.97, 5.92, 6.14 ppm), and the one (C-14) hydroxyl remains free. On chromic acid oxidation, the triacetate (V) furnished the 14-dehydro-triacetate (VI), m.p. 146-147°, whose IR spectrum indicates the formation of a carbonyl in a five-membered ring (ν 1725 cm^{-1}). The chemical shifts of the signals for the (C-17) methyl hydrogens, which are considerably deshielded due to the locations at the hydroxyl-bearing carbons, in the ^1H NMR spectra of G-B, G-B triacetate (V), and 14-dehydro-G-B triacetate (VI) are consistent with those of leucothol B (VII), grayanotoxin II (VIII), and their corresponding derivatives (Table). These findings show that the (C-14) carbinyl group is involved in a bicyclo-

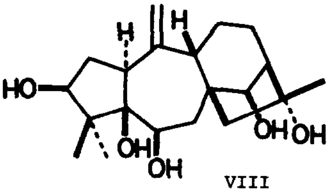
Table ^1H NMR chemical shifts of C-17 methyl hydrogens

	ppm		ppm		ppm
Grayanol[a]	1.52	Leucothol B[a]	1.48	Grayanotoxin II[a]	1.50
1,3,6-Triacetate[b]	1.40	3,5-Diacetate[b]	1.36	3,6-Diacetate[b]	1.33
14-Dehydro-triac.[b]	1.52	14-Dehydro-diac.[b]	1.47	14-Dehydro-diac.[b]	1.43

[a] in C_5D_5N [b] in $CDCl_3$



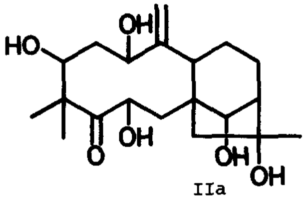
VII



VIII

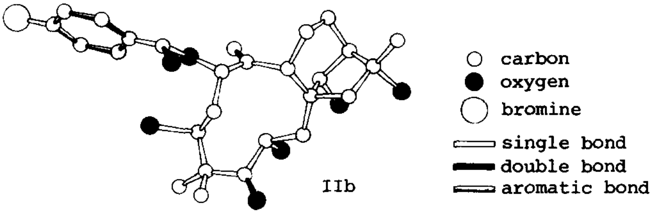
[3.2.1]octane system as in leucothol B and grayanotoxin II, leading to the extension of formula C_1 to C_2.

From the accumulated data together with the circumstantial evidence that G-B coexists with leucothols and grayanotoxins in the same plant, it was thought to be most probable that G-B has the structure IIa. In order to confirm this assumption, G-B was made to react with *p*-bromo-



IIa

benzoyl chloride in pyridine to give the monobenzoate, m.p. 228°, the dibenzoate, and the tribenzoate. Then the monobenzoate was subjected to X-ray crystal structure analysis to reveal that the monobenzoate has the stereostructure (relative configuration and conformation) as shown in IIb.[‡] The negative Cotton effect for the *n*-π* transition ($[\theta]_{302}$ -610) of the C-14 carbonyl



IIb

group, which was claculated by substraction of the CD curve of the triacetate (V) from that of the 14-dehydro-triacetate (VI), is compatible with the Cotton effects exhibited by the CD curves of 14-dehydro-leucothol B 3,6-diacetate5) and 14-dehydro-grayanotoxin II 3,6-diacetate,6) establishing the absolute stereochemistry (II) for G-B.

After the establishment of the stereostructure of G-B, the less stable isomer G-A is most reasonably concluded from its spectral and chemical properties to be the epimer of G-B in regard to C-6 as represented in stereoformula I.

The grayanols have thus been clarified to possess the new carbon skeleton and constitute a unique group of diterpenoids from the viewpoint of biosynthesis.

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† The ^{1}H NMR spectra were determined using a JEOL PS-100 NMR spectrometer and the ^{13}C NMR spectra were measured on a JEOL PS-100 pulsed-Fourier transform NMR spectrometer. Chemical shifts are given in ppm downfield from internal TMS.

‡ The X-ray crystallographic study was performed by Dr. M. Nishikawa *et al.*, Research Laboratories, Takeda Chemical Industries, Ltd., to whom our sincere thanks are due.

1) *cf.*, H. Hikino, S. Koriyama, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull.*, 20, 422 (1972).

2) N. Hamanaka, H. Miyakoshi, A. Furusaki, and T. Matsumoto, *Chemistry Letters*, 779 (1972).

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