Tetrahedron Vol. 29, pp. 773 to 779. Pergamon Press 1973. Printed in Great Britain

STEREOSTRUCTURES OF LEUCOTHOL B AND D, DITERPENOIDS OF *LEUCOTHOE GRAYANA*

H. HIKINO, S. KORIYAMA and T. TAKEMOTO
Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai, Japan

(*Received in Japan* 20 *September* 1972; *Received in the UK for publication* 6 *November* 1972)

Abstract—Two novel diterpenoids, leucothol B and D, have been isolated from the leaves of *Leucothoe grayana* (Ericaceae). Chemical and spectroscopic investigations have shown that leucothol B and D have stereostructures **2** and **3**, respectively, and possess a unique carbon skeleton.

Leucothoe grayana Maximowicz (Ericaceae) is a famous poisonous shrub in Japan, its leaves irritate the mucous membranes and are an effective insecticide for fly larvae. Efforts directed towards the isolation and structure determination of the toxic principles over several decades resulted in the isolation of thirteen toxic diterpenoids of the andromedane skeleton, grayanotoxin I–XIII, having been previously isolated.[1–6] A further survey has revealed the occurrence of other substances which give a green coloration on heating with sulfuric acid spray reagent on thin-layer plates apart from the grayanotoxins which afford purple to brown coloration by the same treatment. An attempt to separate these constituents has resulted in the isolation of three new diterpenoids. During the structural investigation of these diterpenoids, the Hokkaido University group isolated a similar substance from the same plant source, *L. grayana* leaves, and established its stereostructure as in formula **1** by an X-ray crystallographic study.[7] Since one of our diterpenoids appeared to be identical with their compound (**1**), both substances were compared and identified. Meanwhile, it was realised that each group had isolated a few more analogs, in which a pair of substances were further identified. Both groups agreed to adopt the terms leucothol A for the former **1** and leucothol B for the latter. Since our third diterpenoid was not identical with their third compound, leucothol C, the term leucothol D was given. In the present paper, we wish to describe in detail evidence leading to stereoformulas **2** and **3** for leucothol B and D, respectively.*

Leucothol B analysed for $C_{20}H_{32}O_5$ which was substantiated by the appearance of the peak at *m/e* 352 due to M ion in the mass spectrum. The IR and ^{1}H NMR spectra indicate the presence of three tertiary methyls (δ 1·40, 1·48, and 1·50 ppm), a vinylidene (ν_{max} 3040, 1642, and 876 cm^{-1}, and 4·96 and 5·12 ppm), and hydroxyls (ν_{max} 3380 cm^{-1}) three of which are secondary (δ 3·37, 3·94, and 4·52 ppm). In the hope that the ^{13}C NMR spectrum would afford valuable information for structural analysis, the spectrum was measured with the aid of complete proton noise decoupling. Twenty carbon resonances were located in which thirteen aliphatic, five carbinyl, and two unsaturated natures were readily identified from their chemical shifts. The off-resonance decoupled spectrum allowed the assignment of the aliphatic carbon signals to three primary (Me) carbons (δ 23·8, 26·9, and 26·9 ppm), five secondary (methylene) carbons (δ 21·1, 26·0, 29·4, 38·8, and 41·9 ppm), three tertiary (methine) carbons (δ 38·2, 52·1, and 55·3 ppm), and two quaternary carbons (δ 50·9 and 54·7 ppm). Further, the five low-field carbinyl resonances were assigned to three tertiary carbons (δ 76·1, 80·7, and 82·5 ppm), and two quaternary carbons (δ 78·2 and 79·5 ppm) and the two resonances appearing in the unsaturated region attributed to carbons bearing two and no hydrogens (δ 106·4 and 151·7 ppm) by off-resonance decoupling. All the C atoms in the molecule were thus classified.

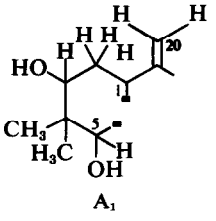
Leucothol B on treatment with acetic anhydride-pyridine formed three acetates: a diacetate (**4**), a triacetate (**5**), and a tetraacetate (**6**). The IR spectrum of **6** still shows retention of an OH (ν_{max} 3460 cm^{-1}), a fact which confirms the aforementioned conclusion derived from the NMR evidence that leucothol B is a penta-ol. All the O atoms in the molecule have thus been accommodated. Since leucothol B has only one ethylenic linkage which was deduced from the above NMR data and corroborated by catalytic hydrogenation of the triacetate (**5**) giving a saturated dihydro-derivative (**7**), leucothol B is a tetracarbocyclic substance.

In order to elucidate the structure, analysis of the ^{1}H NMR spectrum with the aid of double resonance experiments was particularly instructive. Thus, one of the two vinyl hydrogens occurring at 5·12 ppm is long-range coupled to a methine hydrogen at 3·07 ppm which appears as a pair of doublets (*J* 2 and 11 Hz) by coupling with adjacent

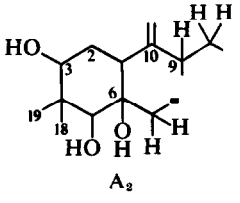
*Part of the material presented herein has been outlined in a preliminary form, *Tetrahedron Letters* 3831 (1972).

methylene hydrogens at ~ 2·0 and ~ 2·5 ppm. The methylene hydrogens are coupled to a carbinyl hydrogen at 3·94 ppm (J 2 and 4 Hz) whose signal is further split by coupling with another carbinyl hydrogen at 3·37 ppm (J 1·5 Hz). The last observation indicates that the two secondary hydroxyls are situated in a 1,2- or 1,3-position. Since intramolecular NOE's were found between the carbinyl hydrogen at 3·94 ppm and the Me hydrogens at 1·40 ppm, the carbinyl hydrogen at 3·94 ppm and the Me hydrogens at 1·50 ppm, the carbinyl hydrogen at 3·37 ppm and the Me hydrogens at 1·40 ppm, and the carbinyl hydrogen at 3·37 ppm and the Me hydrogens at 1·50 ppm, the possibility that the two hydroxyls constitute a 1,2-glycol system is eliminated and it was concluded that a geminal dimethyl group is adjacent to both the secondary carbinol moieties. As the carbinyl hydrogen at 3·37 ppm is coupled to only the carbinyl hydrogen at 3·94 ppm, the second carbon next to the former carbinol group was regarded as quaternary or, in a special case, tertiary. These findings have thus clarified a part structure (the environment of the A-ring) as in formula A_1.

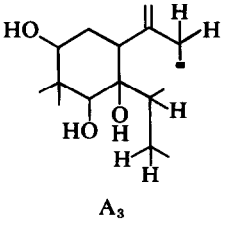
Ozonolysis of leucothol B furnished a norketone (**8**), the IR spectrum of which shows the disappearance of the vinylidene group and instead the formation of a CO group (ν_{max} 1712 cm^{-1}). On treatment with sodium carbonate-sodium hydrogen carbonate in methanol, **8** gave a dehydrated derivative (**9**) whose UV and IR spectra exhibiting maxima at 247 nm (log ϵ 3·88), and 1648 and 1634 cm^{-1}, respectively, are consistent with those (λ_{max} 254 nm (log ϵ 3·89) and ν_{max} 1647 and 1600 cm^{-1}) of the enone (**10**) derived from grayanotoxin II (**11**) *via* the norketone (**12**). These observations together with the lack of a vinyl hydrogen signal in the ^1H NMR spectrum of the anhydro-norketone (**9**) indicate that a fully substituted α,β-unsaturated ketone moiety is present. Further examination of the ^1H NMR spectrum of **9** demonstrates that the C-1 methine hydrogen signal, observed in the spectrum of the parent norketone (**8**), is missing and the C-5 carbinyl hydrogen signal is displaced downfield (−1·7 ppm) in comparison with that of the norketone (**8**). These spectral data indicate the formation of a tetrasubstituted ethylenic linkage (C-1 : C-6) adjacent to C-5 during the dehydration of the norketone (**8**), showing that a tertiary OH is located at C-6 in leucothol B, and that the A-ring is consequently 6-membered. The IR bands of the norketone (**8**) and the anhydro-norketone (**9**), demonstrate that the ring (B) containing the CO is 6- or larger-membered. In the ^1H NMR spectrum of **9**, there are a few more significant signals: a pair of doublets in an AB type at 2·84 and 3·40 ppm (J 18 Hz) and a doublet of doublets at 3·37 ppm (J 5·5 and 9 Hz) which, from their deshielded positions, are thought to originate from allylic methylene and methine hydrogens, respectively.



A_1

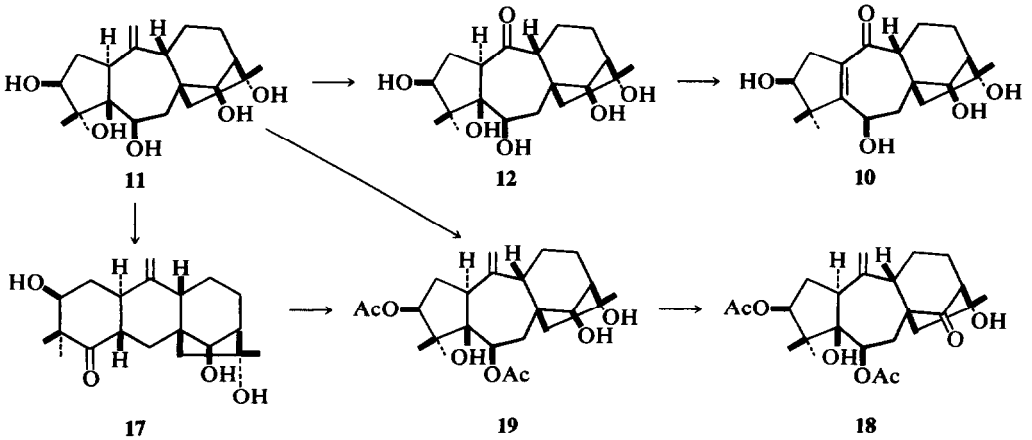


A_2



A_3

■ shows a quaternary carbon

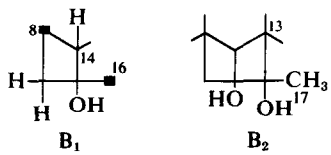


11 **12** **10**

17 **19** **18**

These findings led to the extension of the partial structure A_1 to A_2 or A_3.

In the ^1H NMR spectrum of leucothol B, a couple of doublets due to insulated methylene hydrogens are observed at 2·25 and 2·51 ppm (J 14 Hz) and the former signal is long-range coupled to a carbinyl hydrogen signal at 4·52 ppm. On this basis, the partial structure B_1 was envisaged.



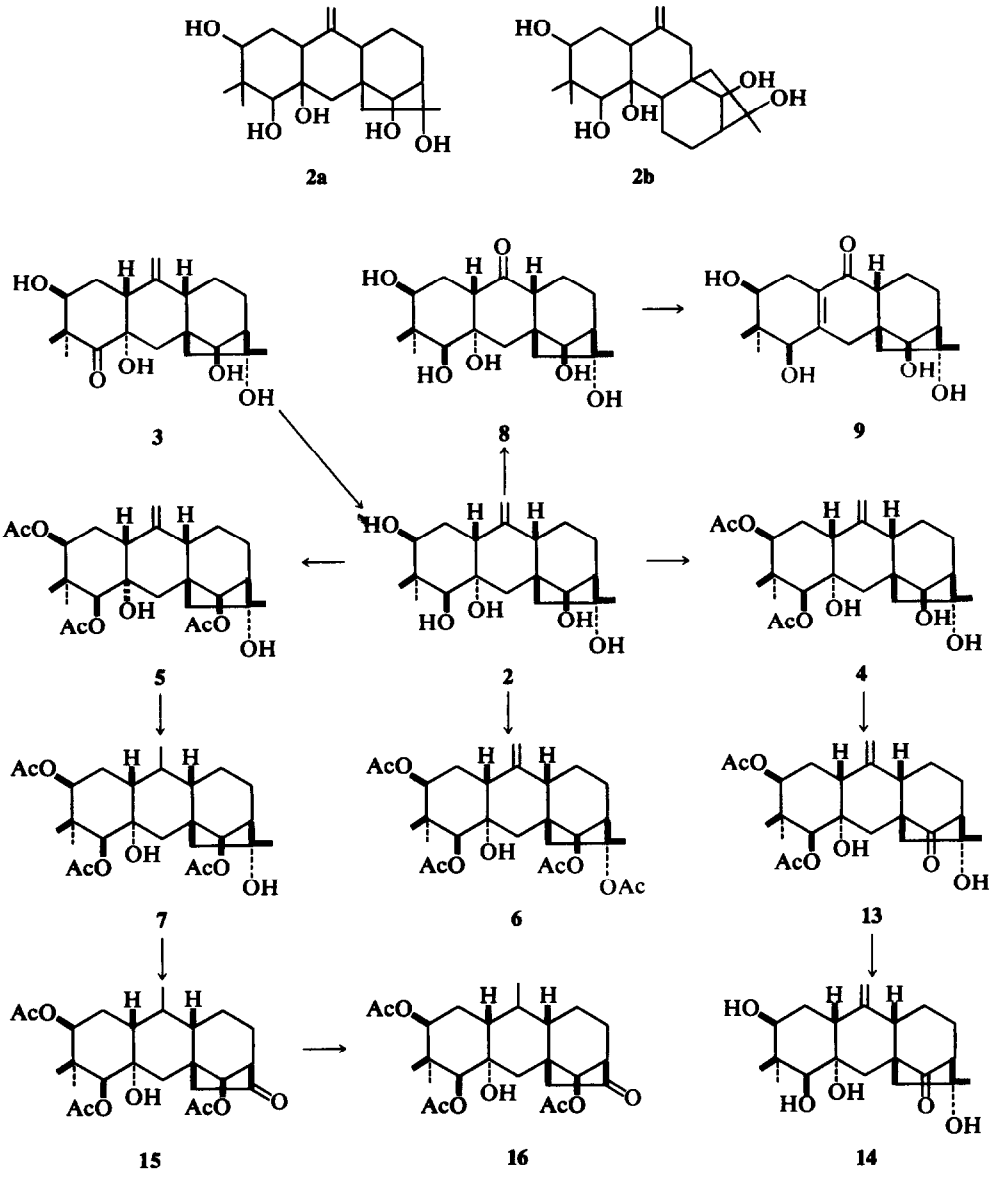
B_1 B_2

■ shows a quaternary carbon

At this point, the esterified patterns of the previous acetates (**4**, **5**, and **6**) should be clarified in order to choose the most suitable derivatives for structure elucidation. Comparison of the ^1H chemical shifts of leucothol B and the diacetate (**4**) shows that on acetylation the resonances for the C-3 and C-5 hydrogens move downfield to 0·86 and 1·27 ppm. The introduction of three acetyls into leucothol B (triacetate **5**) brought about lowfield shifts of the C-3, C-5, and C-14 hydrogen signals by 0·89, 1·28, and 0·56 ppm, respectively. Besides the appearance of the C-3, C-5, and C-14 resonances of the tetraacetate (**6**) in the lower-field region, the resonance for hydrogens of a tertiary Me also underwent a downfield shift of 0·26 ppm on going from the triacetate (**5**) to the tetraacetate (**6**), indicating that an OH on a carbon carrying a tertiary Me was further acetylated in the tetraacetate (**6**).

Chromic acid oxidation of the 3,5-diacetate (**4**) gave the 14-dehydro-derivative (**13**). Although the ring size of the newly formed carbonyl could not be determined immediately from its IR spectrum since the ketonic CO band is obscure due to overlapping of the acetoxyl CO bands, its 5-ring nature was concluded by the IR spectrum of its bisdeacetyl derivative (**14**) showing only a band at 1724 cm^{-1} in the CO region. Treatment of the dihydrotriacetate (**7**) with cupric sulfate in dioxane yielded an anhydro-derivative (**15**) whose spectral properties show the formation of a vinylidene grouping (ν_{max} 1653 and 889 cm^{-1}, and two 1H signals in the region 4·7–5·1 ppm), indicating that the tertiary OH on the Me-bearing carbon was dehydrated to form an exocyclic double bond. Ozonolysis of the anhydro-derivative (**15**) afforded a norketone (**16**). Although the IR spectrum of **16** discloses, in the CO region, a complexed absorption pattern originating from overlapping of a ketonic CO and acetoxyl CO bands, the formation of a CO in a 5-membered ring during the ozonolysis was pointed out by the occurrence of a band at 1740 cm^{-1} as compared with the absorption pattern in the CO region of the parent vinylidene derivative (**15**). Since in leucothol B there are only four quaternary carbons as evidenced by the ^{13}C NMR spectrum, two of which have been assigned to those in the A-ring, one of the remaining two which carries both the Me and the OH must be allotted to C-8 or C-16 in formula B_1. The C-14 carbinyl hydrogen signal in the ^1H NMR spectrum of the anhydro-derivative (**15**) does not exhibit a downfield shift but an upfield shift (+0·17 ppm) relative to that of the parent triacetate (**7**), which excluded the formation of an allylic alcohol moiety during the dehydration of the triacetate (**7**). Therefore, the quaternary carbon bearing both the Me and the OH was assigned to C-16 and not to C-8 which is next to the C-14 carbinol group. The C-14 carbinyl hydrogen signals in the ^1H NMR spectra of leucothol B and its derivatives occur as singlets, a fact which indicates that the second carbon (C-13) adjacent to C-14 is quaternary or tertiary. However, since all the four quaternary carbons in the molecule have already been accommodated, it is consequently tertiary. Formula B_1 is thus extended to B_2. Since the quaternary carbon (C-8) in formula B_2 is deduced to be identical with that as yet unassigned quaternary carbon in formula A_2 or A_3, combination of the two partial formulas led to only two possible structures, **2a** and **2b**, for leucothol B. Provided that leucothol B is a diterpenoid biosynthesized from the common precursor geranyl geraniol, the only probable structure is **2a** which further satisfies the following behaviour of leucothol B.

The next problem to be elucidated is the stereochemistry. In the ^1H NMR spectrum of leucothol B, the coupling constants between the C-1 and C-2 hydrogens (J 2 and 11 Hz) are attributed to axial-equatorial and axial-axial couplings, while those between the C-2 and C-3 hydrogens (J 2 and 4 Hz) indicate the C-3 hydrogen to be equatorially oriented. A long range coupling between the C-3 and C-5 hydrognes (J 1·5 Hz) shows that both the hydrogens are located in a w-arrangement, demonstrating the equatorial nature of the C-5 hydrogen. In agreement with this assignment, intramolecular NOE's observed between the C-3 and C-18, C-3 and C-19, C-5 and C-18, and C-5 and C-19 hydrogens, demonstrate that both the C-3 and C-5 hydrogens are situated in the spatially close relationship to the C-18 and C-19 Me hydrogens. The CD curve of 5-dehydro-leucothol B, leucothol D (**3**) (*vide infra*), exhibits a negative Cotton effect ($[\theta]_{313} - 4250$) which, on inspection of the Dreiding models and the Octant diagrams, indicated the 1(R),6(S)- or 1(S),6(S)-configuration which consequently points to the α-configuration of the C-6 OH in leucothol B regardless of the absolute configuration at C-1. In support of this deduction, the CD curve of leucothol A (**1**) having the 1β(H),6α(H)-arrangement exhibits a negative Cotton effect ($[\theta]_{302} - 12450$), and that of the congener (**17**) possessing the 1α(H),6β(H)-configuration, which was



prepared for comparison, from grayanotoxin II (**11**) by treatment with *p*-toluenesulfonylchloride,[1] shows a positive Cotton effect ($[\theta]_{303}+2050$). Comparison of the CD maximum of 5-dehydro-leucothol B (**3**) with that of leucothol A (**1**) reveals that a bathochromic shift of 11 nm and the decrease of the molecular ellipticity are observed, a fact which suggests that the C-6 OH α to the C-5 CO is axially situated. No consumption of periodate by leucothol B supports the *trans*-diaxial relationship of the C-5 and C-6 hydroxyls. The CD curve of the 14-dehydro-diacetate (**13**) exhibits a negative Cotton effect ($[\theta]_{298}-350$) which is consistent with that ($[\theta]_{300}-2940$) of 14-dehydro-grayanotoxin II 3,6-diacetate (**18**).[5] From this finding, the C-14 carbonyls in both the substances (**13** and **18**) are regarded as being located in a similar environment. The change between the molecular ellipticities of the two Cotton effects may be due to the different effects mediated by the A/B-rings of both the substances of different skeletons. Further, the CD curve of the 17-norketone (**16**) shows a positive Cotton effect ($[\theta]_{299}+4750$). These data establish the absolute configuration of the bicyclo[3.2.1]octane moiety as 8(S), 13(R).[8] The fact that the coupling constant between the C-13 and C-14 hydrogens is very small is explained by the α-configuration of the C-14 hydrogen when the dihedral angle between the C-13 and C-14 hydrogens is nearly 90°. The C-14 CO group in the 14-dehydro-diacetate (**13**) acts to deshield the C-17

Me hydrogens by 0·10 ppm relative to those in the 14-hydroxy-analog (**4**). This downfield shift is in accord with that (0·11 ppm) between grayanotoxin II 3,6-diacetate (**19**) and its 14-dehydro-derivative (**18**), showing the β-configuration of the 17 Me. Supporting this conclusion, the chemical shifts of the C-17 Me hydrogens in the ^{1}H NMR spectra of leucothol B and its derivatives are in good accord with those of grayanotoxin II (**12**) and its corresponding derivatives (Table 1), demonstrating that the two methyls in the substances of different series are situated in a similar environment. Further, the C-17 Me hydrogen signal in the ^{1}H NMR spectrum of the 3,5,14-triacetate (**5**) when compared with that of the 3,5-diacetate (**4**) does not suffer from the acetylation (upfield) shift[9] as is expected if the C-17 OH were α-oriented since the C-14 OH and the C-17 Me is close together in this case. The ORD and CD curves of the 20-norketone (**8**) displaying positive Cotton effects ($a+57$, $[\theta]_{298}+3700$) are similar to those ($a+30$, $[\theta]_{298}+2550$), including the shapes of the curves, of the 20-norketone (**20**) derived from leucothol A (**1**) *via* the dihydro-derivative (**21**). This observation, which points to the like environment of the C-10 carbonyls in both the norketones (**8** and **20**), indicates that the C-1 and C-9 hydrogens are both β-oriented. After the establishment of the absolute stereochemistry at the A/B ring junction, both the C-3 and C-5 hydroxyls, whose stereochemical natures have been determined only as equatorial, must be allocated the β-configurations.

From the accumulated data, it is concluded that leucothol B possesses the stereostructure **2**.

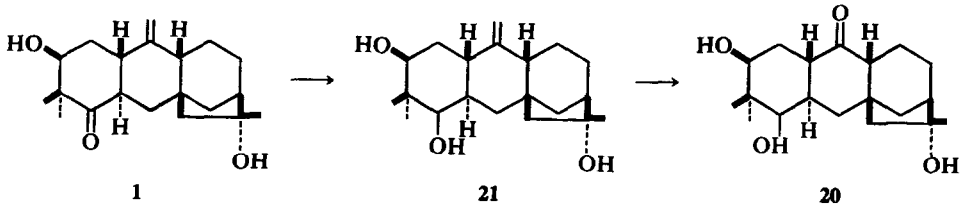
Leucothol D has the composition $C_{20}H_{30}O_5$ as substantiated by the mass spectrum which shows M ion peak at *m/e* 350. The IR and ^{1}H NMR spectra indicate that leucothol D possesses three tertiary methyls (δ 1·44, 1·49, and 1·57 ppm), a vinylidene (ν_{max} 3100, 1651, and 878 cm^{-1}, and δ 4·98 and 5·12 ppm), a CO in a 6- or larger-membered ring (ν_{max} 1707 cm^{-1}), and hydroxyls (ν_{max} 3460 and 3360 cm^{-1}) two of which are secondary (δ 4·15 and 4·47 ppm). These spectral properties of leucothol D resemble those of leucothol B (**2**) except that the C-5 carbinyl hydrogen signal, observed in the spectrum of the latter, is absent and instead a CO band at 1707 cm^{-1} is present. On this basis, leucothol D is considered to be the 5-dehydro derivative of leucothol B. Then hydride reduction of leucothol D was carried out to yield two epimeric dihydro derivatives, in which the less polar one was identified as leucothol B(**2**). Whereupon it follows that leucothol D is represented by stereoformula **3**.

After the establishment of the absolute stereostructures of the leucothols, we were interested in the biogenesis of these diterpenoids having the novel skeleton. If the leucothols had the 1α(H), 6β(H or OH)-arrangement, they are regarded as

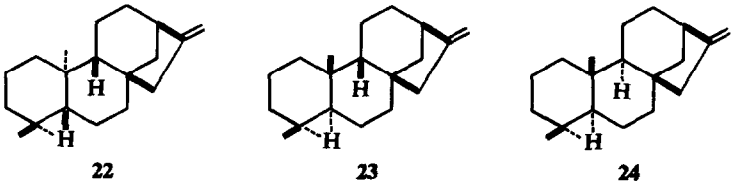
Table 1. ^{1}H NMR chemical shifts of C-17 methyl hydrogens

	ppm		ppm
Leucothol B (**2**)[a]	1·48	Grayanotoxin II (**11**)[a]	1·50
3,5-Diacetate (**4**)[b]	1·36	3,6-Diacetate (**19**)[b]	1·33
3,5,14-Triacetate (**5**)[b]	1·36	3,6,14-Triacetate[b]	1·35
3,5,14,16-Tetraacetate (**6**)[b]	1·62	3,6,14,16-Tetraacetate[b]	1·64
14-Dehydro-diacetate (**13**)[b]	1·47	14-Dehydro-diacetate (**18**)[b]	1·43

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



1 **21** **20**



22 **23** **24**

being A-homo-B-nor-grayanotoxins. Since the grayanotoxins are thought to be A-nor-B-homo-kaurenoids, the leucothols may thus be concluded to be double rearranged products derived from the intermediate (−)-kaurene (**22**) in this case. However, the leucothols have, in fact, the 1β(H), 6α(H or OH)-arrangement and, therefore, they are considered to be biosynthesized from the unknown intermediate (**23**), the 9-epimer of phyllocladene (**24**), by a double rearrangement in the *A*/*B* ring.

Concerning the biological properties, leucothol B and D do not irritate mucous membranes. The toxicity is considered to be very low, the LD_{50} of leucothol B being larger than 100 mg/kg (*i.p.*) in the mouse.

EXPERIMENTAL

M.p.s are uncorrected. The IR spectra were recorded in KBr disks. The ^1H NMR spectra were determined using a Varian HA-100 and a Hitachi R-20 NMR spectrometer and the ^{13}C NMR spectrum was measured on a JEOL PS-100 pulsed-Fourier transform NMR spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS. Abbreviations: s = singlet, d = doublet, m = multiplet, dd = doublet of doublets, and br = broad. TLC was carried out on silica gel G plates, developing solvents being shown in parentheses.

Isolation of leucothol A, B, and D. The dried leaves (10 kg) of *Leucothoe grayana* Maximowicz (Ericaceae) were extracted 3 times with refluxing MeOH (90 l each) for 5 hr (each extraction). The combined MeOH soln was concentrated to give an extract (2·3 kg) whose AcOEt soluble portion was chromatographed over alumina. Fractions eluted with AcOEt were combined and re-chromatographed over silica gel.

Elution with benzene-AcOEt (5:1) and crystallization from MeOH–AcOEt gave **1** as colorless needles (25 mg). m.p. 265–267°; ORD (*c* 0·070, dioxane): $[\phi]_{316}^{trough}$ −9740, $[\phi]_{273}^{peak}$ +10040, CD (*c* 0·070, dioxane): $[\theta]_{302}$ −12440. MS *m/e*: 318 (M^+); IR ν_{max} cm^{-1}: 3530, 3400 (OH); 3090, 1640, 882 (vinylidene), 1700 (cyclohexanone); ^1H NMR (C_5D_5N, 100 MHz): 3 H s at 1·16 ($C_{(18)}\underline{H}_3$), 3 H s at 1·43 ($C_{(19)}\underline{H}_3$), 3 H s at 1·52 ($C_{(17)}\underline{H}_3$), 1 H dd at 2·73 (J = 2·5, 13, $C_{(1)}\underline{H}$), 1 H dd at 4·14 (J = 2·5, 3, $C_{(3)}\underline{H}$), two 1 H s's at 4·88, 5·06 ($C_{(20)}\underline{H}_2$).

Elution with benzene-AcOEt (2:1) and crystallization from MeOH–AcOEt yielded **3** as colorless needles (30 mg). m.p. 259–260°; ORD (*c* 0·156, dioxane): $[\phi]_{333}^{trough}$ −6100, $[\phi]_{287}^{peak}$ +5800, CD (*c* 0·156, dioxane): $[\theta]_{313}$ −4260; MS *m/e*: 350 (M^+); IR ν_{max} cm^{-1}: 3460, 3360 (OH), 3100, 1651, 878 (vinylidene), 1707 (cyclohexanone), ^1H NMR (C_5D_5N, 100 MHz): 3 H s at 1·44 ($C_{(17)}\underline{H}_3$), 3 H s at 1·49 ($C_{(18)}\underline{H}_3$), 3 H s at 1·57 ($C_{(19)}\underline{H}_3$), 1 H dd at 3·17 (J = 1·5, 12·5, $C_{(1)}\underline{H}$), 1 H dd at 4·15 (J = 3·5, 2, $C_{(3)}\underline{H}$), 1 H s at 4·47 ($C_{(14)}\underline{H}$), two 1 H s's at 4·98, 5·12 ($C_{(20)}\underline{H}_2$).

Elution with benzene-AcOEt (1·1) and crystallization from MeOH–AcOEt afforded **2** as colorless needles (0·50 g). m.p. 257–258°; MS *m/e*: 352 (M^+); IR ν_{max} cm^{-1}: 3380 (OH), 3040, 1642, 876 (vinylidene); ^1H NMR (C_5D_5N, 100 MHz): 3 H s at 1·40 ($C_{(18)}\underline{H}_3$), 3 H s at 1·48 ($C_{(17)}\underline{H}_3$), 3 H s at 1·50 ($C_{(19)}\underline{H}_3$), two 1 H d's at 2·25, 2·51 (J = 14, $C_{(15)}\underline{H}_2$), 1 H dd at 3·07 (J = 2, 11, $C_{(1)}\underline{H}$), 1 H ddd at 3·94 (J = 2, 11, 1·5, $C_3\underline{H}$), 1 H d at 3·37 (J = 1·5, $C_{(5)}\underline{H}$), 1 H s at 4·52 ($C_{(14)}\underline{H}$), two 1 H s's at 4·96, 5·12 ($C_{(20)}\underline{H}_2$); ^{13}C NMR (C_5D_5N, 25 MHz): described in the text. (Found: C, 68·15; H, 9·15. $C_{20}H_{32}O_5$ requires: C, 68·06; H, 9·12%).

Acetylation of leucothol B with acetic anhydride in pyridine. Leucothol B (80 mg) was dissolved in Ac_2O (1 ml) and pyridine (2 ml) and set aside at room temp for 2 days. Upon isolation in the usual manner, the product was chromatographed over silica gel (10 g).

Elution with benzene-AcOEt (5:1) furnished **6** as colorless needles (30 mg). m.p. 245–246°; IR ν_{max} cm^{-1}: 3460 (OH), 3100, 1649, 890 (vinylidene), 1716, 1230 (acetoxyl); ^1H NMR ($CDCl_3$, 60 MHz): 3 H s at 0·84 ($C_{(18)}\underline{H}_3$), 3 H s at 1·23 ($C_{(19)}\underline{H}_3$), 3 H s at 1·62 ($C_{(17)}\underline{H}_3$), four 3 H s's at 1·92, 2·00, 2·02, 2·09 ($C\underline{H}_3COO$), 1 H d at 4·60 (J = 1·5, $C_{(5)}\underline{H}$), 1 H m at 4·82 ($C_{(3)}\underline{H}$), 1 H s at 4·86 ($C_{(14)}\underline{H}$), two 1 H s's at 4·91, 5·00 ($C_{(20)}\underline{H}_2$).

Fractions elution with benzene-AcOEt (2:1) were combined and submitted to preparative TLC ($CHCl_3$—MeOH=20:1) to give **5** as an amorphous mass (20 mg); IR ν_{max} cm^{-1}: 3510 (OH), 3090, 1694, 890 (vinylidene), 1738, 1720, 1230 (acetoxyl); ^1H NMR ($CDCl_3$, 60 MHz). 3 H s at 0·83 ($C_{(18)}\underline{H}_3$), 3 H s at 1·23 ($C_{(19)}\underline{H}_3$), 3 H s at 1·36 ($C_{(17)}\underline{H}_3$), three 3 H s's at 2·03, 2·07, 2·11 ($C\underline{H}_3COO$), 1 H br s at 4·65 ($C_{(5)}\underline{H}$), 1 H m at 4·83 ($C_{(3)}\underline{H}$), 1 H s at 5·08 ($C_{(14)}\underline{H}$), two 1 H s's at 4·94, 5·01 ($C_{(20)}\underline{H}_2$), and **4** as colorless needles (from AcOEt). m.p. 178–179°; IR ν_{max} cm^{-1}: 3430 (OH), 3110, 1649, 890 (vinylidene), 1729, 1707, 1226 (acetoxyl); ^1H NMR ($CHCl_3$, 60 MHz): 3 H s at 0·84 ($C_{(18)}\underline{H}_3$), 3 H at 1·28 ($C_{(19)}\underline{H}_3$), 3 H s at 1·36 ($C_{(17)}\underline{H}_3$), two 3 H s's at 2·03, 2·10 ($C\underline{H}_3COO$), 1 H s at 4·04 ($C_{(14)}\underline{H}$), 1 H br s at 4·64 ($C_{(5)}\underline{H}$), 1 H m at ~4·8 ($C_{(3)}\underline{H}$), 2 H s at 4·89 ($C_{(20)}\underline{H}_2$).

Hydrogenation of leucothol B 3,5,14-triacetate over Adams' catalyst in methanol. The triacetate (**5**) (10 mg) in MeOH (10 ml) was hydrogenated over Pt_2O in the presence of a small amount of $HClO_4$. After isolation, the product was purified by preparative TLC ($CHCl_3$—MeOH=15:1) to afford **7** as an amorphous mass (8 mg); IR ν_{max} cm^{-1}: 3460 (OH), 1723, 1233 (acetoxyl); ^1H NMR ($CHCl_3$, 60 MHz): 3 H s at 0·82 ($C_{(18)}\underline{H}_3$), 3 H d at 1·26 (J = 5, $C_{(20)}\underline{H}_3$), 3 H s at 1·28 ($C_{(19)}\underline{H}_3$), 3 H s at 1·37 ($C_{(17)}\underline{H}_3$), 1 H d at 4·43 (J = 1·5, $C_{(5)}\underline{H}$), 1 H m at 4·80 ($C_{(3)}\underline{H}$), 1 H s at 5·50 ($C_{(14)}\underline{H}$).

Ozonolysis of leucothol B. Leucothol B (55 mg) was dissolved in AcOH (10 ml) and a stream of ozonized O_2 passed through at 15° for 0·5 hr. After removal of the solvent, the product was extracted with AcOEt. Working up in the usual manner and crystallization from MeOH–AcOEt gave **8** as colorless needles (35 mg). m.p. 230–231°; ORD (*c* 0·129, dioxane): $[\phi]_{312}^{peak}$ +2510, $[\phi]_{272}^{trough}$ −3200, CD (*c* 0·129, dioxane): $[\theta]_{298}$ +3716. IR ν_{max} cm^{-1}: 3430 (OH), 1712 (cyclohexanone); ^1H NMR (C_5H_5N, 60 MHz): 3 H s at 1·38 ($C_{(18)}\underline{H}_3$), 6 H s at 1·47 ($C_{(19)}\underline{H}_3$ and $C_{(17)}\underline{H}_3$), 1 H d at 3·47 (J = 1·5, $C_{(5)}\underline{H}$), 1 H m at 3·92 ($C_{(3)}\underline{H}$), 1 H s at 4·37 ($C_{(14)}\underline{H}$).

Alkali dehydration of the 20-norketone from leucothol B. The 20-norketone **8** (32 mg) in MeOH (3 ml) was treated with *M*/5 Na_2CO_3 (0·1 ml) and *M*/5 $NaHCO_3$ (1 ml) on a steam bath for 1 hr. The mixture was diluted with water and extracted with AcOEt. After work up and crystallization from MeOH–AcOEt, **9** was obtained as colorless needles (16 mg). m.p. 264–266°; UV λ_{max}^{MeOH} nm (log ϵ): 247 (3·88); IR ν_{max} cm^{-1}: 3390 (OH), 1648, 1634 (cyclohexenone); ^1H NMR (C_5D_5N, 100 MHz): 3 H s at 1·30 ($C_{(18)}\underline{H}_3$), 3 H s at 1·35 ($C_{(19)}\underline{H}_3$), 3 H s at 1·49 ($C_{(17)}\underline{H}_3$), two 1 H d's at 1·96, 2·24 (J = 15, $C_{(15)}\underline{H}_2$), two 1 H d's at 2·84, 3·40 (J = 18, $C_{(7)}\underline{H}_2$), 1 H dd at 3·77 (J = 5·5, 9, $C_{(9)}\underline{H}$), 1 H s at 3·96 ($C_{(14)}\underline{H}$), 1 H m at 4·15 ($C_{(3)}\underline{H}$); ^1H NMR ($C_5D_5N(D_2O)$, 100 MHz): 1 H br s at 5·25 ($C_{(5)}\underline{H}$).

Alkali dehydration of the 20-norketone from grayano-

toxin II. The 20-norketone (**12**) (70 mg), derived from grayanotoxin II (**11**), in MeOH (4 ml) was heated on a steam bath with *M*/5 Na_2CO_3 (0·1 ml) and *M*/5 $NaHCO_3$ (1 ml) for 1 hr. BuOH extraction and crystallization from MeOH–AcOEt afforded **10** as colorless needles (25 mg) m.p 255–256°; UV λ_{max}^{MeOH} nm (log ϵ). 254 (3·89). IR ν_{max} cm^{-1}. 3500, 3430, 3370 (OH), 1647, 1600 (cycloheptenone).

Chromic acid oxidation of leucothol B 3,5-*diacetate.* The diacetate **4** (15 mg) in AcOH (3 ml) was allowed to react with $K_2Cr_2O_7$ (50 mg) at room temp for 2 hr. The mixture was diluted with water and extracted with AcOEt. The product was purified by preparative TLC (light petroleum–AcOt–MeOH=8:5:1), and crystallization from MeOH–AcOEt to give **13** as colorless needles. m.p. 188–190°; ORD (*c* 0·200, MeOH) $[\phi]_{308}^{trough}$ −63, $[\phi]_{288}^{peak}$ +610, CD (*c* 0·200, MeOH) $[\theta]_{298}$ −360, MS *m/e* 374 (M^+-60), 314 (M^+-120); IR ν_{max} cm^{-1} 3450 (OH), 3110, 1649, 890 (vinylidene), 1740, 1735, 1710, 1260, 1232 (cyclopentanone, acetoxyl), ^1H NMR ($CDCl_3$, 60 MHz): 3 H s at 0·84 ($C_{(18)}\underline{H}_3$), 3 H s at 1·31 ($C_{(19)}\underline{H}_3$), 3 H s at 1·47 ($C_{(17)}\underline{H}_3$), two 3 H s's at 2·02, 2·08 ($C\underline{H}_3COO$), 1 H d at 4·69 (J = 1·5, $C_{(5)}\underline{H}$), 1 H m at ~ 4·8 ($C_{(3)}\underline{H}$), 2 H s at 4·83 ($C_{(20)}\underline{H}_2$).

Alkaline hydrolysis of 14-*dehydroleucothol B* 3,5-*diacetate* The diacetate (**13**) (2 mg) in 2% ethanolic KOH (1 ml) was left standing at room temp for 24 hr. Isolation and purification by preparative TLC (AcOEt) gave **14** as an amorphous mass (1 mg); IR ν_{max} cm^{-1}: 3400, 3360 (OH), 3100, 1650, 891 (vinylidene), 1724 (cyclopentanone).

Dehydration of dihydroleucothol 3,5,14-*triacetate with cupric sufate in dioxane.* The triacetate **7** (12 mg) in dioxane (3 ml) was heated under reflux with $CuSO_4$ (150 mg) for 5 hr Isolation and crystallization from AcOEt afforded **15** as colorless needles (7 mg). m.p 248–249°, IR ν_{max} cm^{-1} 3440 (OH), 1653, 889 (vinylidene), 1720, 1701, 1230 (acetoxyl); ^1H NMR ($CDCl_3$, 60 MHz): 3 H s at 0·82 ($C_{(18)}\underline{H}_3$), 3 H s at 1·30 ($C_{(19)}\underline{H}_3$), 3 H d at 1·27 (J = 5, $C_{(20)}\underline{H}_3$), 6 H s at 2·04 ($C\underline{H}_3COO$), 3 H s at 2·08 ($C\underline{H}_3COO$), 1 H d at 4·43 (J = 1·5 $C_{(5)}\underline{H}$), 1 H s at 5·33 ($C_{(14)}\underline{H}$), 3 H m at 4·7–5·1 ($C_{(3)}\underline{H}$ and $C_{(17)}\underline{H}_2$).

Ozonolysis of 16-*anhydro-dihydroleucothol B* 3,5,14-*triacetate.* The triacetate **15** (6 mg) in AcOH (3 ml) was ozonized at 15° for 0·5 hr. The product was isolated and purified by preparative TLC (benzene-AcOEt = 2:1) to yield **16** as an amorphous mass (3 mg); ORD (*c* 0·057, MeOH). $[\phi]_{332}^{peak}$ +2560, $[\phi]_{280}^{trough}$ −2180, CD (*c* 0·057, MeOH): $[\theta]_{299}$ +4750; IR ν_{max} cm^{-1}: 3460 (OH), 1740 (cyclopentanone), 1720, 1702, 1235 (acetoxyl).

Rearrangement of grayanotoxin II with p-*toluenesulfonyl chloride in pyridine.* Grayanotoxin II (**11**) (200 mg) in pyridine (2 ml) was heated with *p*-TsCl (120 mg) at 100° for 7 hr. The mixture was diluted with water and extracted with AcOEt. The extract was worked up in the customary way and chromatographed over silica gel (8 g). Elution with benzene-AcOEt (5:1) gave **17** as colorless powder (30 mg); ORD (*c* 0·142, dioxane): $[\phi]_{324}^{peak}$ +830, $[\phi]_{278}^{trough}$ −2580, CD (*c* 0·142, dioxane): $[\theta]_{303}$ +2050; MS *m/e*: 334 (M^+), IR ν_{max} cm^{-1}: 3410 (OH), 3090, 1641, 886 (vinylidene), 1693 (cyclohexanone); ^1H NMR (C_5D_5N, 100 MHz): 3 H s at 1·40 ($C_{(18)}\underline{H}_3$), 6 H s at 1·47 ($C_{(19)}\underline{H}_3$ and $C_{(17)}\underline{H}_3$), 1 H dd at 3·04 (J = 14, 4·5, $C_{(1)}\underline{H}$), 1 H dd at 3·88 (J = 10·5, 4·5, $C_{(3)}\underline{H}$), 1 H s at 4·16 ($C_{(14)}\underline{H}$), two 1 H s's at 4·73, 4·99 ($C_{(20)}\underline{H}_2$).

Hydride reduction of leucothol A. Leucothol A (10 mg) in THF (3 ml) was treated with LAH_4 (20 mg) at room temp for 2 hr. Dilution with water, extraction with AcOEt, purification by prepartive TLC (benzene-AcOEt=1:2) furnished **21** as an amorphous mass (4 mg); IR ν_{max} cm^{-1}: 3410 (OH), 1635, 880 (vinylidene).

Ozonolysis of the dihydroleucothol A. Compound **21** (4 mg) in AcOH (2 ml) was ozonized at 15° for 0·5 hr. Isolation and purification by preparative TLC (benzene-AcOEt=1:2) afforded **20** as an amorphous mass (2 mg); ORD (*c* 0·057, dioxane): $[\phi]_{312}^{peak}$ +1420, $[\phi]_{272}^{trough}$ −1620, CD (*c* 0·057, dioxane): $[\theta]_{296}$ +2550; IR ν_{max} cm^{-1}: 3400 (OH), 1695 (cyclohexanone).

Hydride reduction of leucothol D. Leucothol D (5 mg) in THF (3 ml) was treated with LAH_4 (10 mg) at room temp for 1 hr. The product was isolated and subjected to preparative TLC (AcOEt). The less polar fraction having the R_f 0·60 was crystallized from MeOH–AcOEt to furnish the dihydroleucothol D as colorless needles (2 mg). m.p. 253–255°, MS *m/e*: 352 (M^+); IR ν_{max} cm^{-1}. 3430 (OH), 3110, 1642, 870 (vinylidene) The identity with leucothol B (**2**) was confirmed by mixed m.p., and MS and IR comparison

Acute toxicity of leucothol B. Five male mice of dd strain weighing about 20 g were used. For administration, leucothol B was suspended in a concentration corresponding to 10 mg/ml physiological saline containing 3% gumi arabicum and an aliquot (0·2 ml) of the soln (100 mg/kg body weight) was injected intraperitoneally into each mouse. No effect was observed during 48 hr.

Acknowledgements—We wish to thank Mr. T. Ohta, this Laboratory, for colloboration in preliminary experiments. Thanks are also due to Analytical Laboratory, Department of Chemistry, this University, for the (100 MHz) ^1H NMR spectra, to JEOL Ltd. for the ^{13}C NMR spectra, and to Analytical Laboratory, this Institute, for the (60 MHz) ^1H NMR and mass spectra, and elemental analysis.

Addendum—Immediately before the submission of this paper, structure elucidation of leucothol B by the Hokkaido University group has been announced in which they have arrived at the same conclusion about its stereostructure as our own (N Hamanaka, H. Miyakoshi, A. Furusaki, and T. Matsumoto, *Chemistry Letters* 787 (1972)).

REFERENCES

[1]H. Kakisawa, T. Kozima, M. Yanai, and K. Nakanishi, *Tetrahedron* **21**, 3091 (1965).
[2]H. Hikino, M. Ogura, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull. Tokyo* **18**, 1071 (1970).
[3]T. Okuno, N. Hamanaka, H. Miyakoshi, and T. Matsumoto, *Tetrahedron* **26**, 4765 (1970).
[4]H. Hikino, N. Shoji, S. Koriyama, T. Ohta, Y. Hikino, and T. Takemoto, *Chem. Pharm. Bull. Tokyo* **18**, 2358 (1970).
[5]H. Hikino, T. Ohta, S. Koriyama, Y. Hikino, and T. Takemoto, *Ibid.* **19**, 1289 (1971).
[6]H. Hikino, S. Koriyama, T. Ohta, and T. Takemoto, *Ibid.* **20**, 422 (1972).
[7]N. Hamanaka, T. Okuno, H. Miyakoshi, A. Furusaki, and T. Matsumoto, *Abstract of the 25th Annual Meeting of the Chemical Society of Japan* p 1241, Hiratsuka, April (1972).
[8]W. Klyne, *Tetrahedron* **13**, 29 (1961).
[9]Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okanoto, and K. Tsuda, *Chem Pharm. Bull. Tokyo* **10**, 338 (1962)