Terpenoids. LI.[1] Structures of Antitumor Diterpenoids, Trichorabdals A—E, Isolated from *Rabdosia trichocarpa*

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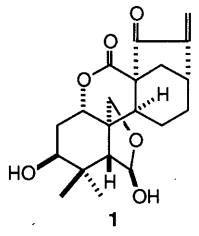
The structures of five B-seco-kaurene type diterpenoids, trichorabdals A—E, isolated from *Rabdosia trichocarpa* were determined by chemical and physical means.

Keywords diterpenoid; trichorabdal A; trichorabdal B; trichorabdal C; trichorabdal D; trichorabdal E; kaurene type; X-ray analysis; antitumor activity

Among the plants of the genus *Rabdosia* (Labiatae), *Rabdosia trichocarpa* (MAXIM.) HARA is a treasury of diterpenoids of kaurene and 6,7-seco-kaurene type, among which enmein (**1**) was the first to be isolated in 1958.[2-4] In the course of our studies of biologically active diterpenoids of *Rabdosia* plants, we reinvestigated the constituents of *Rabdosia trichocarpa* collected in Hyogo prefecture and isolated five new 6,7-seco-kaurene type diterpenoids, trichorabdals A (**2**), B (**3**), C (**4**), D (**5**), and E (**6**). Antitumor activity of those diterpenoids has been reported.[5] Here, we present a full account of the work reported in preliminary communications.[6-9]

Structures of Trichorabdals Usual isolation procedure including chromatographic separation (see Experimental) afforded trichorabdals A—E (**2**—**6**) and the known diterpenoid, longikaurin D (**7**).[9] Five oxygen atoms of trichorabdal A (**2**) were assigned as follows: an α-methylene cyclopentanone moiety [λ_{max}^{EtOH} nm (ε): 232 (8500); ν_{max}^{KBr} cm^{-1}: 1690, 1640; ^{1}H-NMR δ: 6.05 (1H, br s), 5.35 (1H, br s); ^{13}C-NMR δ: 200.7 (s, C-15), 150.6 (s, C-16), 117.1 (t, C-17), a δ-lactone (ν_{max}^{KBr} cm^{-1}: 1745), an aldehyde group [ν_{max}^{KBr} cm^{-1}: 2820, 2730, 1725; ^{1}H-NMR δ: 10.03 (1H, d, J=3 Hz)], and a secondary hydroxyl group [ν_{max}^{KBr} cm^{-1}: 3600, 3450; ^{1}H-NMR δ: 4.60 (1H, m, C<u>H</u>–OH, shifted to δ 5.37 on acetylation), ^{13}C-NMR δ: 64.9 (d)]. Trichorabdal A (**2**) was recognized to have the 6,7-seco-kaurene skeleton, and the location of the oxygen functional groups was assigned as shown in formula **2**. The characteristic downfield shift of α-H at C-14 indicates an α-configuration for the hydroxyl group at C-11. Upon acetylation, trichorabdal A (**2**) afforded a monoacetate **8**, which is identical with the product obtained by the periodate oxidation of the known longikaurin E (**9**),[10] confirming the structure **2** for trichorabdal A (*ent*-11β-hydroxy-6,7,15-trioxo-7,20-epoxy-6,7-seco-16-kaurene).

A close inspection of the spectral data for trichorabdal B leads to the straightforward assignment of structure **3**. Periodate oxidation of longikaurin D (**7**) afforded a compound whose identity with trichorabdal B (**3**) was demonstrated by a comparison of spectral data and by mixed melting point determination. This confirmed the absolute structure of trichorabdal B (**3**) to be as *ent*-19-acetoxy-11β-hydroxy-6,7,15-trioxo-7,20-epoxy-6,7-seco-16-kaurene. Treatment of trichorabdal B (**3**) with sodium hydroxide in methanol at 0 °C provided **10** with a hitherto unknown molecular skeleton. An X-ray structural analysis of the compound determined the structure to be that shown in



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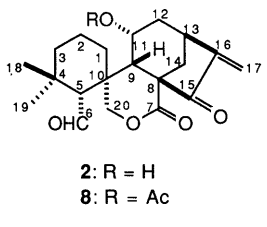
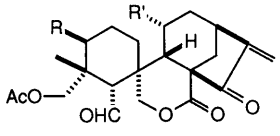
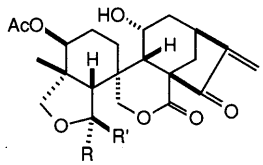


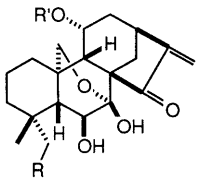
2: R = H
8: R = Ac



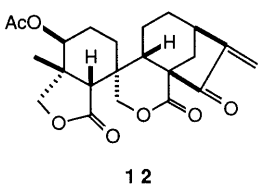
3: R = H, R' = OH
4: R = OH, R' = H
5: R = R' = OH



6a: R = H, R' = OH
6b: R = OH, R' = H
15: R = H, R' = OAc
16a: R = H, R' = OMe
16b: R = OMe, R' = H



7: R = OAc, R' = H
9: R = H, R' = Ac



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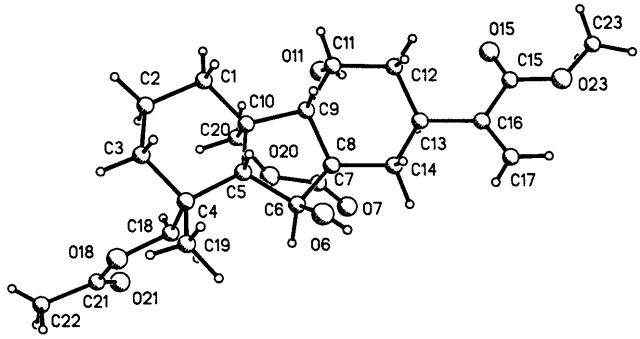


Fig. 1. Drawing of **10** with Atoms Represented by Spheres of Arbitrary Size

Fig. 1. A retro-Claisen condensation followed by an aldol condensation, shown in Chart 1, can account for the transformation to this novel skeleton.

Trichorabdal C (**4**) is an isomer of trichorabdal B (**3**). The basic structure was deduced from the spectral data. The only problem involved the location and configuration of the secondary hydroxyl group. An axial orientation of the hydroxyl group was indicated from the coupling pattern (t, $J=3$ Hz), which was consistent with the hydroxyl group being located at either C-1 or C-3. On treatment with acetic acid at 90 °C under nitrogen, trichorabdal C (**4**) furnished a diacetate **11**. The ^{1}H-NMR signal of 19-H_2 in this compound appeared at a higher field (δ 3.67 and 3.80, ABq, $J=$ 8 Hz) than in trichorabdal C (**4**) (see Table I), indicating a deacetylation of the acetoxyl group at C-19. Disappearance of the aldehyde signal and appearance of a new signal at δ 6.78 (1H, d, $J=5.0$ Hz) indicated the formation of a hemiacetal acetate between C-6 and C-19. The coupling constant of this signal eliminates the possibility that the acetal involves C-6 and C-20, as seen in enmein (**1**).[11] Formation of the γ-lactone **12** on the Jones oxidation of **11** confirms the 5-membered hemiacetal acetate moiety in **11**. Overall transformation from trichorabdal C (**4**) to the diacetate **11** involves the migration of the acetyl group from the oxygen at C-19 to the secondary hydroxyl group in question followed by the formation of the hemiacetal **13** and acetoxylation of the corresponding oxonium ion **14** from the less hindered β- side, as shown in Chart 2. Migration of the acetyl group could not take place if the secondary hydroxyl group were located at C-1. The total structure of trichorabdal C (**4**), including the conformation of ring A, was determined by an X-ray crystallographic analysis to be *ent*-19-acetoxy-3α-hydroxy-6,7,15-trioxo-7,20-epoxy-6,7-seco-16-kaurene.

Trichorabdal D (*ent*-19-acetoxy-3α,11β-dihydroxy-6,7,15-trioxo-7,20-epoxy-6,7-seco-16-kaurene) (**5**) possesses two secondary hydroxyl groups. Comparison of the ^{1}H-NMR data of trichorabdal D (**5**) with those of trichorabdal B (**3**) (see Table I) indicates the location of a hydroxyl group at C-11 with an α-configuration. The position of the remaining hydroxyl group was assigned to be at C-3 because, upon treatment with acetic acid at 90 °C, the same type of acetyl migration as observed with trichorabdal C (**4**) took place to afford the diacetate **15**. The stereochemistry of the diacetate **15**, including the conformation of ring A as well as the configuration of the acetoxyl group at C-3, was

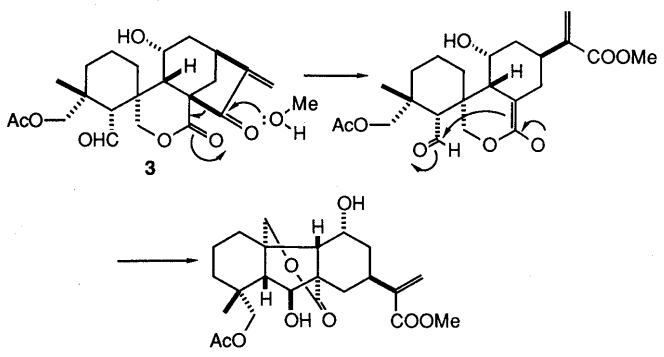


Chart 1

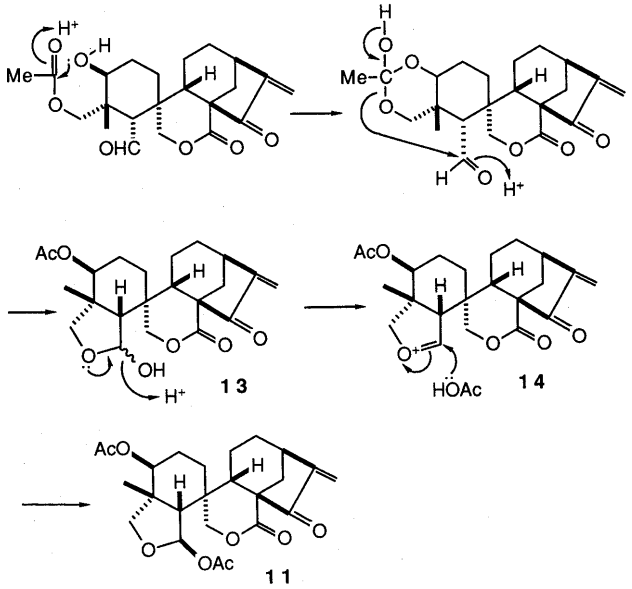


Chart 2

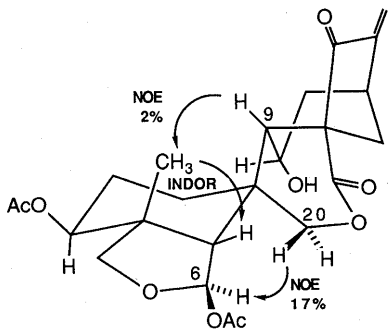


Fig. 2. NOE of Trichorabdal C Acetate **15**

TABLE I. Pertinent ^{1}H-NMR Data[a] for Trichorabdals A—D (**2**—**5**)

Trichorabdal	3-H	5-H	CHO	11-H	13-H	14α-H	17-H_2	19-H_2	20-H_2	CH_3	OAc
A (**2**)[b]	c)	2.90	10.03	4.60	3.12	3.45	6.05 (s)		4.71, 5.1	0.95 (s)	
		(d, 3)[d]	(d, 3)	(m)	(dd, 10, 4)	(d, 12)	5.35 (s)		(ABq, 12)	1.00 (s)	
B (**3**)	c)	3.50	10.19	4.50	3.11	3.50	6.05 (s)	4.03, 4.16	4.50, 5.15	1.19 (s)	2.00
		(d, 4)	(d, 4)	(m)	(dd, 8, 4)	(d, 12)	5.46 (s)	(ABq, 12).	(ABq, 12)		
C (**4**)	3.80	3.20	10.09	c)	2.88	c)	5.93 (s)	4.28, 4.46	4.98, 5.04	1.44 (s)	1.95
	(t, 3)	(d, 3.5)	(d, 3.5)		(dd, 8, 4)		5.31 (s)	(ABq, 12)	(ABq, 12)		
D (**5**)	4.20	3.54	10.22	4.58	3.14	3.50	6.06 (s)	4.27, 4.52	4.30, 5.16	1.40 (s)	2.02
	(m)	(d, 4)	(d, 4)	(m)	(dd, 10, 4)	(d, 12)	5.48 (s)	(ABq, 12)	(ABq, 12)		

a) Measured in C_5D_5N at 60 °C unless otherwise stated. *b*) At 40 °C. *c*) Can not be assigned because of overlapping with other signals. *d*) Numbers in parenthesis are coupling constants in Hz.

supported by the ^{1}H-NMR data. The nuclear Overhauser enhancement (NOE) is shown in Fig. 2. Ring A of **15** exists in a chair conformation with C-9 axial and C-20 equatorial. The ^{1}H-NMR signal of H-3 at δ 5.16 (dd, J=11, 4 Hz) indicated a β-equatorial orientation of the acetoxyl group at this position. Thus, the β-configuration of the hydroxyl group at C-3 in trichorabdal D (**5**) was established.

Acetylation of trichorabdal E (**6**) with acetic anhydride in pyridine afforded the same acetate **15** as that derived from trichorabdal D (**5**). Mild acid hydrolysis of **15** regenerated trichorabdal E (**6**), confirming the structure of trichorabdal E (**6**). Each resolvable signal in the ^{1}H-NMR spectrum of trichorabdal E (**6**) appeared as a pair with a ratio of 2:1 when measured in $CDCl_3$. The ratio changed to 5:2 in $CDCl_3$/dimethyl sulfoxide (DMSO-d_6). This indicates that trichorabdal E (**6**) exists as a mixture of **6a** (*ent*-3α-acetoxy-6α,11β-dihydroxy-7,15-dioxo-6,19 : 7,20-diepoxy-6,7-seco-16-kaurene) and **6b** (*ent*-3α-acetoxy-6β,11β-dihydroxy-7,15-dioxo-6,19 : 7,20-diepoxy-6,7-seco-16-kaurene) in solution, and each isomer, **6a** and **6b**, was trapped as the methyl acetal **16a** and **16b**.

Conformation of Ring A in Trichorabdals Figures 3 and 4 are drawings of trichorabdals B (**3**) and C (**4**) using the coordinates from the X-ray analysis.[12] The relative configurations of the two molecules are the same; however, the A ring chair conformations differ significantly. In trichorabdal B (**3**), C-19 and C-20 occupy equatorial sites while C-9 and C-18 occupy axial sites on one face of the molecule with the aldehyde C-6 axial on the other. In trichorabdal C (**4**), C-9, C-6 and C-19 are equatorial with O-3 being axial on one face and C-19 and C-20 axial on the opposite. It is difficult to rationalize these differences in conformation as arising from steric interactions, and both conformers might be expected to occur in solution. The signal of the proton at C-3 in trichorabdal C (**4**) appears as a triplet at δ 3.80 with a coupling constant of 3 Hz, suggesting an equatorial orientation of H-3. This is consistent with the X-ray data, as illustrated in Fig. 4.

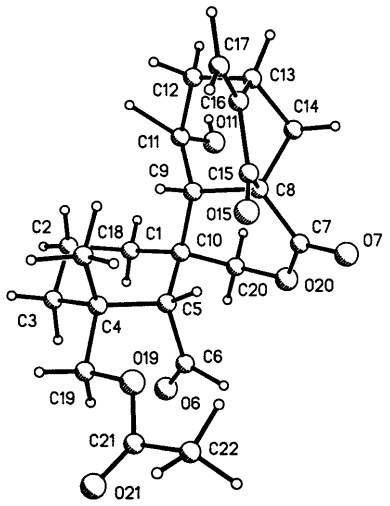


Fig. 3. Drawing of Trichorabdal B (**3**) with Atoms Represented by Spheres of Arbitrary Size

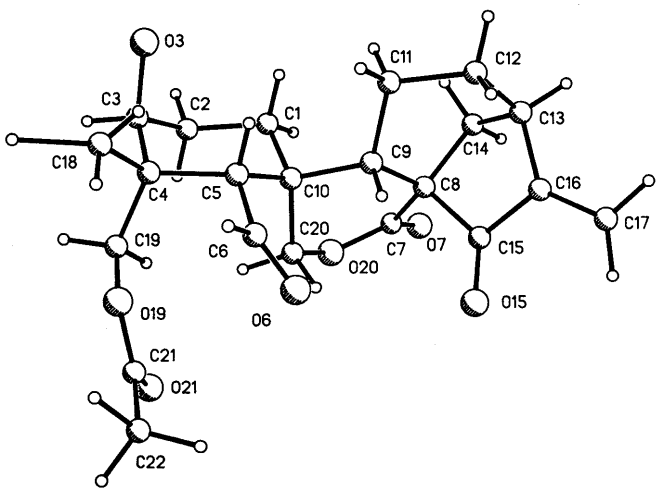
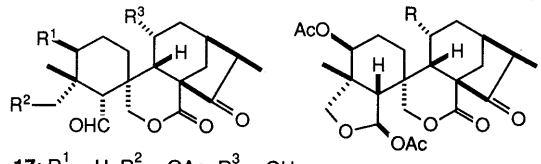


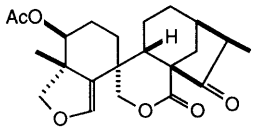
Fig. 4. Drawing of Trichorabdal C (**4**) with Atoms Represented by Spheres of Arbitrary Size

Absolute Configurations of Trichorabdals The absolute configuration of trichorabdal B was established to be as shown in **3** by the direct relationship with longikaurin D (**7**) and by the circular dichroism (CD) curve of dihydrotrichorabdal B (**17**). The CD curve of dihydrotrichorabdal A (**18**) confirmed the absolute configuration of trichorabdal A (**2**). Dihydrotrichorabdal C (**19**) in refluxing acetic acid undergoes the same type of transformation as that for trichorabdal C (**4**) to provide **20** along with **21**. The CD curve of **20** determined the absolute stereochemistry of trichorabdal C to be as shown in **4**. The absolute configurations of trichorabdals D (**5**) and E (**6**) were deduced from the CD curve of the dihydro derivative **22** of trichorabdal E acetate **15**.



17: R^1 = H, R^2 = OAc, R^3 = OH
18: R^1 = R^2 = H, R^3 = OH
19: R^1 = OH, R^2 = OAc, R^3 = H

20: R = H
22: R = OH



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Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were measured with Hitachi EPS-3 or JASCO UVIDEC 610C spectrometer and infrared (IR) spectra were measured with a JASCO DS=70 G spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a JEOL GX-400 and JEOL FX-100, and chemical shifts are recorded in δ (ppm) with tetramethylsilane (TMS) as an internal reference. Electron impact mass spectra (EI-MS) were measured with a JEOL DX-300 mass spectrometer. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter and CD spectra were measured with JASCO J-20 (ORD/CD) spectrometer. Unless otherwise noted, the reaction products were purified by column chromatography on Kieselgel 60 (E. Merck, 70—230 mesh) or thin layer chromatography (TLC) on 20×20 cm glass plates coated with a 0.25 mm layer of Kiesel gel 60 F_{254} (E. Merck).

Isolation The crushed dry leaves (1.5 kg) of *Rabdosia trichocarpa* (Maxim) Hara collected in Hyogo prefecture were refluxed with MeOH. The methanolic extract was concentrated *in vacuo*. The residue was dissolved in water and the solution was extracted with AcOEt. The residue of this AcOEt extract (50 g) was chromatographed on a silica gel column

(500 ml/fraction). The results are summarized below.

fraction No.	residue (g)	solvent	main diterpenoids of the fraction
30—37	(3.9)	CH_2Cl_2	trichorabdal A
38—46	(15.8)	CH_2Cl_2–Me_2CO (7:3)	trichorabdals B and C
49—57	(9.4)	CH_2Cl_2–Me_2CO (7:3)	longikaurin D
65—66	(0.8)	Me_2CO	trichorabdal D
73—75	(4.0)	Me_2CO	trichorabdals E, F and G

Isolation of Trichorabdal A (2) The residue of fractions 30—37 of the 1st column chromatography was re-chromatographed with CH_2Cl_2 as an eluent to afford TLC-pure trichorabdal A, which was recrystallized from MeOH to give **2** as colorless needles, mp 198—201 °C. $[\alpha]_D^{25}$ −63.9° (c=0.01, EtOH). UV λ_{max}^{EtOH}: nm (ε): 232 (8500). IR ν_{max}^{KBr} cm^{-1}: 3600, 3450, 2820, 2730, 1745, 1725, 1690, 1640, 1275. ^{1}H-NMR: see Table I. ^{13}C-NMR (FX 100, C_5D_5N, at 60 °C) δ: 204.5 (d, C-6), 200.7 (s, C-15), 169.5 (s, C-7), 150.6 (s, C-16), 117.1 (t, C-17), 70.7 (t, C-20), 64.9 (d, C-11), 60.7 (d, C-5), 56.7 (s, C-8), 47.8 (d, C-9), 42.7 (s, C-10), 40.3 (s, C-4), 42.0 (t, C-12), 35.2 (d, C-13), 34.2 (t, C-3), 31.5 (t, C-1), 28.4 (t, C-14), 32.3 (q, C-18), 26.0 (q, C-19), 18.6 (t, C-2). *Anal.* Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.01; H, 7.80.

Isolation of Trichorabdal B (3) The residue of fractions 38—46 of the 1st column chromatography was re-chromatographed. Elution with CH_2Cl_2–Me_2CO (9:1) afforded trichorabdal B, which was recrystallized from MeOH to give **3** as colorless needles, mp 160—161 °C. $[\alpha]_D^{25}$ −120.3° (c=1.0, EtOH). UV λ_{max}^{EtOH} nm (ε): 232 (9100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 2820, 2720, 1740, 1710, 1640, 1220. ^{1}H-NMR: see Table I. ^{13}C-NMR (FX 100, C_5D_5N, at 60 °C) δ: 203.0 (d, C-6), 200.4 (s, C-15), 170.0, 169.9 (s, C-7 and –O<u>C</u>OCH$_3$), 150.6 (s, C-16), 117.5 (t, C-17), 71.4, 70.8 (each t, C-19 and C-20), 65.0 (d, C-11), 57.9 (d, C-5), 56.4 (s, C-8), 46.2 (d, C-9), 35.1 (d, C-13), 42.0 (t, C-12), 41.5 (s, C-10), 38.5 (s, C-4), 33.7 (t, C-3), 32.3 (t, C-1), 28.0 (t, C-14), 26.7 (q, C-18), 20.3 (q, –OCO<u>C</u>H$_3$), 18.1 (t, C-2). *Anal.* Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.23; H, 7.08.

Isolation of Trichorabdal C (4) In another isolation procedure from 1 kg of dry leaves, 15.9 g of a fraction containing trichorabdals B (**3**) and C (**4**) was separated by essentially the same method as described above. Crude trichorabdal B (**3**) was removed from this fraction by crystallization from AcOEt, then a portion of the residue (1.7 g) was subjected to flash column chromatography with AcOEt–petroleum ether (1:1) (25 ml/fraction) to give trichorabdal C (**4**) (500 mg), which was recrystallized from AcOEt–*n*-pentane to afford colorless prisms (30 mg), mp 146.5—149 °C. $[\alpha]_D^{25}$ +31.5° (c=1.0, EtOH). UV λ_{max}^{EtOH} nm (ε): 235 (9000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2750, 1745, 1715, 1640, 1230. ^{1}H-NMR: see Table I. ^{13}C-NMR (FX 100, C_5D_5N) δ: 203.1 (d, C-6), 202.4 (s, C-15), 170.5, 170.1 (each s, C-7 and –O<u>C</u>OCH$_3$), 150.6 (s, C-16), 117.8 (t, C-17), 69.6, 68.7 (each t, C-19 and C-20), 68.9 (d, C-3), 57.8 (s, C-8), 55.6 (d, C-5), 44.7 (d, C-9), 44.0 (s, C-10), 41.4 (s, C-4), 35.3 (d, C-13), 30.0, 29.5, 25.8, 18.9, 17.3 (all t, C-1, C-2, C-11, C-12 and C-14), 24.2 (q, C-18), 20.4 (q, –OCO<u>C</u>H$_3$). *Anal.* Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.36; H, 7.00.

Isolation of Trichorabdal D (5) The residue from fractions 65 and 66 of the 1st column chromatography was recrystallized several times from MeOH to give trichorabdal D (**5**) as colorless needles (59 mg), mp 213—215 °C. $[\alpha]_D^{25}$ −89.2° (c=0.01, EtOH). UV λ_{max}^{EtOH} nm (ε): 230 (10200). IR ν_{max}^{KBr} cm^{-1}: 3550, 3470, 2750, 1745, 1730, 1710, 1705, 1640, 1240. ^{1}H-NMR: see Table I. ^{13}C-NMR (FX 100, C_5D_5N, at 60 °C) δ: 203.6 (d, C-6), 200.0 (s, C-15), 170.9, 170.2 (each s, C-7 and –OCO<u>C</u>H$_3$), 150.6 (s, C-16), 118.0 (t, C-17), 72.1, 70.9 (each t, C-19 and C-20), 69.6 (d, C-3), 65.1 (d, C-11), 56.4 (d, C-5), 55.4 (d, C-8), 45.1 (d, C-9), 35.1 (d, C-13), 43.4 (s, C-10), 41.1 (s, C-4), 42.1 (t, C-12), 33.0 (t, C-1), 27.4 (t, C-14), 24.9 (t, C-2), 20.8, 20.4 (each q, C-18 and –OCO<u>C</u>H$_3$). *Anal.* calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 62.61; H, 6.85.

Isolation of Trichorabdal E (6) The residue from fractions 73—75 of the 1st column chromatography was chromatographed with AcOEt–petroleum ether (1:1). The crude crystals (372 mg) were recrystallized from $CHCl_3$–MeOH to afford 6 as colorless prisms (85 mg), mp 291 °C (dec.). $[\alpha]_D^{25}$ −98.4° (c=0.02, EtOH). UV λ_{max}^{EtOH} nm (ε): 229 (7700). IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1720, 1695, 1640, 1235. *Anal.* Calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 63.10; H, 6.85. Resolvable ^{1}H-NMR signals are listed in Table II.

Acetylation of Trichorabdal A (2) Trichorabdal A (**2**) (32 mg) was dissolved in Ac_2O–C_5H_5N and left overnight at room temperature. Usual workup afforded a crude residue (50 mg), which was chromatographically purified to give trichorabdal A acetate (**8**), amorphous solid. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2850, 2750, 1740, 1715, 1640, 1230. ^{1}H-NMR (FX 100, $CDCl_3$) δ: 1.07, 1.12 (each 3H, s, 4-Me$_2$, 2.10 (3H, s, OAc), 2.50 (2H, d, J=4 Hz, 5-H and m, 12-H), 2.67 (1H, d, J=12 Hz, 14α-H), 3.12 (1H, dd, J=9, 4 Hz, 13-H), 4.46, 4.76 (each 1H, ABq, J=12 Hz, 20-H$_2$) 5.37 (1H, m, 11-H), 5.55, 6.09 (each 1H, s, 17-H$_2$), 9.86 (1H, d, J=4 Hz, 6-H). High MS Calcd for $C_{22}H_{28}O_6$: 388.189. Found: 388.187.

Table II. ^{1}H-NMR Chemical Shifts of Trichorabdal E at 400 MHz in $CDCl_3$

Ratio	-CH$_3$	-Ac	14-H	19-H$_2$	20-H$_2$	3-H	6-H
2	1.06	2.03	3.31	3.53, 4.01	4.41, 4.79	5.38	5.60
:			(d, 12)	(ABq, 9)	(ABq, 11)	(dd, 12, 4)	(dd, 4, 2)
1	0.99	2.02	3.23	3.61, 3.73	(—), 4.90	4.90	5.47
			(d, 11)	(—)	(br d, 12)	(—)	(t, 6)

δ ppm, numbers in parenthesis are coupling constants in Hz.

Oxidation of Longikaurin D (7) Sodium periodate (1.58 g) was stirred in water (0.5 ml) and MeOH (20 ml), and then longikaurin D (**7**) (150 mg) was added. The mixture was stirred for 7.5 d at room temperature, then poured into 5% hydrochloric acid and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford a residue (149 mg). Purification by column chromatography followed by recrystallization gave trichorabdal B (**3**) (15 mg).

Methanolysis of Trichorabdal B (3) To a solution of trichorabdal B (**3**) (52 mg) in MeOH (25 ml) distilled water (20 ml) and 0.1% NaOH (5 ml) were added under ice-cooling. After being stirred for 5 min, the reaction mixture was poured into ice-water, acidified with 5% HCl, and extracted with AcOEt. After usual treatment, the extract was concentrated *in vacuo*, purified, and recrystallized from MeOH to afford **10** (26 mg), mp 214.5—217 °C. $[\alpha]_D^{25}$ −190.7° (c=0.002, EtOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3550, 1740, 1730, 1160. ^{1}H-NMR (FX 100, C_5D_5N) δ: 1.45 (3H, s, 4-Me), 1.99 (3H, s, OAc), 1.91 (1H, d, J=7 Hz, 5-H), 3.58 (3H, s, OMe), 4.34, 5.14 (each 1H, ABq, J=11.5 Hz, 19-H$_2$), 4.81 (1H, d, J=7 Hz, 6-H), 4.56, 5.14 (each 1H, ABq, J=11.5 Hz, 20-H$_2$), 5.64, 6.20 (each 1H, s, 17-H$_2$), 6.68, 7.03 (each 1H, br s, OH×2, disappeared with D_2O). ^{13}C-NMR (FX 100, C_5D_5N) δ: 176.4 (s, C-7), 170.9 (s, C-7, –O<u>C</u>OCH$_3$), 167.6 (s, C-15), 146.0 (s, C-16), 123.2 (t, C-17), 73.8 (d, C-6), 72.4, 68.3 (each t, C-19 and C-20), 65.4 (d, C-11), 64.9 (d, C-5), 54.3 (d, C-9), 52.5 (s, C-8), 51.6 (q, -OMe), 43.0, 36.7 (each s, C-4 and C-10), 40.9, 37.5, 32.5, 32.1 (all t, C-1, C-3, C-12 and C-14), 31.3 (d, C-13), 28.09 (q, C-18), 20.6 (q, C-18, –OCO<u>C</u>H$_3$), 19.5 (t, C-2). *Anal.* Calcd for $C_{23}H_{32}O_8$: C, 63.28; H, 7.39. Found: C, 63.10; H, 7.58.

The Diacetate 11 Trichorabdal C (**4**) (320 mg) was dissolved in AcOH (5 ml) and stirred at 90 °C under an N_2 atmosphere for 10 h. The reaction mixture was evaporated to dryness, and the residue was purified by column chromatography with $CHCl_3$–Me_2CO (9:1) to afford **11** (130 mg), which was recrystallized from AcOEt to give prisms (104 mg), mp 218.5—220.5 °C. IR ν_{max}^{KBr} cm^{-1}: 1740, 1730, 1720, 1700, 1638, 1245. ^{1}H-NMR (FX 100, C_5D_5N) δ: 1.17 (3H, s, 4-Me), 2.04 (3H, s, 3-OAc), 2.18 (3H, s, 6-OAc), 2.40 (1H, d, J=12 Hz, 14α-H), 2.62 (1H, dd, J=5, 2 Hz, 5-H), 2.94 (1H, dd, J=9, 4 Hz, 13-H), 3.67, 3.80 (each 1H, ABq, J=8 Hz, 19-H$_2$), 3.92, 4.27 (each 1H, dd, J=12, 2 Hz, and d, J=12 Hz, 20-H$_2$), 5.08 (1H, dd, J=11, 4 Hz, 3-H), 5.43, 6.09 (each 1H, s, 17-H$_2$), 6.78 (1H, d, J=5 Hz, 6-H). *Anal.* Calcd for $C_{24}H_{30}O_8$: C, 64.56; H, 6.77. Found: C, 64.38; H, 6.73.

The Jones Oxidation of 11 A solution of **11** (62 mg) in acetone (10 ml) was treated with Jones reagent under ice-cooling. The reaction mixture was stirred for 45 min at room temperature, then isopropyl alcohol was added and the mixture was poured into ice-water, and extracted with CH_2Cl_2. Usual work up yielded 40 mg of crude material. Separation by preparative TLC afforded the product **12** (7 mg) and the starting material (5 mg). The product was recrystallized with AcOEt–hexane, mp 145—148 °C (dec.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1750, 1735, 1720, 1640, 1230. ^{1}H-NMR (FX 100, $CDCl_3$) δ: 1.18 (3H, s, 4-Me), 2.07 (3H, s, OAc), 3.14 (1H, br d, J=8 Hz, 13-H), 3.80, 3.97 (each 1H, ABq, J=8 Hz, 19-H$_2$), 4.08 (1H, ABq, J=11 Hz, 20-H$_a$), 4.80 (2H, m, 3-H and 20-H$_b$), 5.56, 6.10 (each 1H, s, 17-H$_2$). High MS Calcd for $C_{22}H_{26}O_7$: 402.168. Found: 402.168.

The Diacetate 15 Trichorabdal D (**5**) (138 mg) was dissolved in AcOH (10 ml) and stirred at 90 °C under an N_2 atmosphere for 7 h. The reaction mixture was evaporated to dryness, and the residue was purified by column chromatography to afford **15** (59 mg). Recrystallization from $CHCl_3$–MeOH gave **15** as colorless needles, mp 229 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3600, 3400, 1750, 1735, 1720, 1695, 1640, 1230. ^{1}H-NMR (FX 100,

C_5D_5N) δ: 1.16 (3H, s, 4-Me), 2.03 (3H, s, 3-OAc), 2.20 (3H, s, 6-OAc), 2.84 (1H, dd, J=6, 2 Hz, 5-H), 3.10 (1H, dd, J=8, 4 Hz, 13-H), 3.60 (1H, d, J=11.5 Hz, 14α-H), 3.74, 3.82 (each 1H, ABq, J=8 Hz, 19-H_2), 4.54 (1H, t, J=3 Hz, 11-H), 4.00, 5.26 (each 1H, dd, J=11.5, 2 Hz and d, J=11.5 Hz, 20-H_2), 5.16 (1H, dd, J=11, 4 Hz, 3-H), 5.49, 6.14 (each 1H, s, 17-H_2), 6.88 (1H, d, J=5.4 Hz, 6-H), 7.00 (1H, d, J=4 Hz, OH, disappeared with D_2O). *Anal.* Calcd for $C_{24}H_{30}O_9$: C, 62.32; H, 6.54. Found: C, 62.24; H, 6.76.

Acetylation of Trichorabdal E (6) Trichorabdal E (**6**) (0.8 g) was dissolved in Ac_2O–C_5H_5N and left at room temperature overnight. Usual extractive work up with AcOEt afforded 1.1 g of crude material, which was purified by column chromatography to afford trichorabdal E acetate (**15**) (172 mg). Recrystallization from $CHCl_3$–MeOH gave colorless flakes (84 mg), mp 229—231 °C. IR ν_{max}^{KBr} cm^{-1}: 3600, 3400, 1750, 1735, 1720, 1695, 1640, 1230. ^{1}H-NMR (FX 100, C_5D_5N) δ: 1.17 (3H, s, 4-Me), 2.05, 2.22 (each 3H, s, OAc×2), 2.86 (1H, dd, J=5.5, 2 Hz, 5-H), 3.10 (1H, dd, J=8, 4 Hz, 13-H), 3.64 (1H, d, J=12 Hz, 14α-H), 3.80, 3.83 (each 1H, ABq, J=9 Hz, 19-H_2), 4.05 (1H, dd, J=12, 2 Hz, 20-H_a), 4.54 (1H, m, 11-H), 5.19 (1H, dd, J=12, 4 Hz, 3-H), 5.32 (1H, d, J=12 Hz, 20-H_b), 5.50, 6.16 (each 1H, s, 17-H_2), 6.92 (1H, d, J=5.5 Hz, 6-H), 7.07 (1H, d, J=3.5 Hz, OH, disappeared with D_2O). *Anal.* Calcd for $C_{24}H_{30}O_9$: C, 62.32; H, 6.54. Found: C, 62.05; H, 6.56.

The Methyl Acetals 16a and 16b Trichorabdal E (**6**) (61 mg) was dissolved in MeOH (30 ml) with 5% HCl (30 drops) and stirred at room temperature for 15 h. The reaction mixture was concentrated and poured into ice-water. Extractive workup with AcOEt followed by preparative TLC with $CHCl_3$–acetone afforded the methyl acetal **16a** (10 mg) and **16b** (8 mg). **16a**: mp 260—261 °C (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 3430, 1740, 1735, 1685, 1640, 1230. ^{1}H-NMR (GX 400, C_5D_5N–$CDCl_3$=1:3) δ: 1.01 (3H, s, 4-Me), 1.98 (3H, s, OAc), 3.12 (1H, dd, J=8.5, 4 Hz, 13-H), 3.38 (3H, s, OMe), 3.60 (1H, d, J=12 Hz, 14α-H), 3.57 (2H, s, 19-H_2), 3.85, 5.13 (each 1H, ABq, J=11 Hz, 20-H_2), 4.43 (1H, m, 11-H), 4.98 (1H, dd, J=12, 4 Hz, 3-H), 5.02 (1H, d, J=5.5 Hz, 6-H), 5.45, 6.06 (each 1H, s, 17-H_2). High MS Calcd for $C_{23}H_{30}O_8$: 434.194. Found: 434.194. **16b**: mp 280 °C (dec.) (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 3510, 1740, 1735, 1710, 1635, 1235. ^{1}H-NMR (GX 400, C_5D_5N–$CDCl_3$=1:3) δ: 1.11 (3H, s, 4-Me), 1.97 (3H, s, OAc), 3.15 (1H, dd, J=9, 5 Hz, 13-H), 3.29 (3H, s, OMe), 3.54 (1H, d, J=13 Hz, 14α-H), 3.55, 3.94 (each 1H, ABq, J=8.5 Hz, 19-H_2), 4.18 (1H, d, J=11 Hz, 20-H_a), 4.35 (1H, m, 11-H), 5.08 (1H, d, J=11 Hz, 20-H_b), 5.10 (1H, d, J=4.5 Hz, 6-H), 5.32 (1H, dd, J=11, 4 Hz, 3-H), 5.46, 6.06 (each 2H, s, 17-H_2). High MS Calcd for $C_{23}H_{30}O_8$ (M^+): 434.194. Found: 434.190.

Hydrogenation of Trichorabdals A (2), B (3), C (4) and E Acetate (15) Each material in an appropriate solvent (**2**, 23.9 mg in 20 ml of AcOEt; **3**, 150 mg in 30 ml of MeOH; **4**, 140 mg in 30 ml of AcOEt; **15**, 30 mg in 5 ml of AcOEt) was hydrogenated over 10% Pd–carbon (10 mg for **2**, 60 mg for **3** and **4**, 5 mg for **15**) under atmospheric pressure of hydrogen for 1 d except for **3** (16 h) to afford the corresponding dihydro derivative **18** (colorless crystals, 19.6 mg), **17** (crystalline residue, 159 mg), **19** (colorless crystals, 132 mg), or **22** (crude crystals, 26 mg), respectively.

Dihydrotrichorabdal A (**18**): mp 146—148.5 °C (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 3600, 3420, 2850, 2755, 1761, 1712, 1232. CD (c=0.10, MeOH) $[\theta]^{25}$ (nm): −1040 (305) (negative maximum). ^{1}H-NMR (FX 100, $CDCl_3$) δ: 1.01, 1.16 (each 3H, s, 4-Me_2), 1.10 (3H, d, J=7 Hz, 16-Me), 2.55 (1H, d, J=7 Hz, 16-H), 2.64 (1H, d, J=5 Hz, 5-H), 2.97 (1H, br d, J=11 Hz, 14α-H), 4.23 (1H, dd, J=4.5 Hz, 11-H), 4.55 (1H, br m, 20-H_a), 4.84 (1H, d, J=11.5 Hz, 20-H_b), 9.84 (1H, d, J=5 Hz, 6-H). *Anal.* Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.70, H, 7.95.

Dihydrotrichorabdal B (**17**): mp 145—146.5 °C (from ether). IR ν_{max}^{KBr} cm^{-1}: 3430, 2830, 2730, 1738, 1720, 1240. CD (c=0.10, MeOH) $[\theta]^{25}$ (nm): −4570 (310) (negative maximum). ^{1}H-NMR (FX 100, C_5D_5N, 60 °C) δ: 1.06 (3H, d, J=6.5 Hz 16-Me), 1.14 (3H, s, 4-Me), 1.96 (3H, s, OAc), 3.17 (1H, d, J=4 Hz, 5-H), 2.29, 3.33 (each 1H, d, and br d, J=12 Hz, 14-H_2), 4.09, 4.24 (each 1H, ABq, J=11 Hz, 19-H_2), 4.37 (1H, t, J=4 Hz, 11-H), 4.66, 4.92 (each 1H, ABq, J=11 Hz, 20-H_2), 6.20 (1H, br s, OH, disappeared with D_2O), 10.12 (1H, d, J=4 Hz, 6-H).

Dihydrotrichorabdal C (**19**): mp 160—162 °C (from AcOEt). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2850, 2750, 1745, 1720. ^{1}H-NMR ($CDCl_3$) δ: 1.11 (3H, d, J=6.9 Hz, 17-H_3), 1.19 (3H, s, CH_3), 2.07 (3H, s, OAc), 2.82 (1H, d, J=3.9 Hz), 3.65 (1H, m), 4.07, 4.18 (each 1H, ABq, J=11.7 Hz), 4.56, 4.69 (each 1H, ABq, J=11.7 Hz), 9.86 (1H, d, J=4.0 Hz). ^{13}C-NMR δ: 10.3 (q, C-17), 16.5 (t), 18.6 (t), 19.1 (t), 19.1 (t), 20.6 (q, OCO<u>C</u>H_3), 23.2 (q, C-18), 25.1 (t), 32.5 (d), 32.6 (t), 40.1 (s), 43.1 (s), 44.1 (d), 48.9 (d), 54.8 (d), 57.2 (s), 68.2, 69.9 (each t, C-19 and C-20), 69.0 (d, C-3), 170.0, 170.5 (each s, C-7 and O<u>C</u>OCH$_3$), 201.7 (d, C-6), 216.0 (s, C-15). *Anal.* Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 64.86; H, 7.38.

Dihydrotrichorabdal E Acetate (**22**): mp 222—224 °C (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 3510, 1745, 1732, 1695, 1245. CD (c=0.10, MeOH) $[\theta]^{25}$ (nm): −3470 (305) (negative maximum). ^{1}H-NMR (FX 100, $CDCl_3$) δ: 1.05 (3H, s, 4-Me), 1.15 (3H, d, J=7 Hz, 16-Me), 2.03 (3H, s, OAc), 2.16 (3H, s, 6-OAc), 2.60 (1H, d, J=7 Hz, 16-H), 3.31 (1H, br d, J=13 Hz, 14α-H), 3.68 (2H, s, 19-H_2), 4.33 (1H, m, 11-H), 3.76 (1H, dd, J=12, 2 Hz, 20-H_a), 4.78 (1H, d, J=12 Hz, 20-H_b), 4.86 (1H, dd, J=10, 4 Hz, 3-H), 6.40 (1H, d, J=5.5 Hz, 6-H). *Anal.* Calcd for $C_{24}H_{32}O_9$: C, 62.05; H, 6.94. Found: C, 61.96; H, 6.81.

Acetolysis of Dihydrotrichorabdal C (19) Dihydrotrichorabdal C (**19**) (132 mg) was dissolved in AcOH and refluxed under an N_2 atmosphere for 2.5 h. The reaction mixture was evaporated to dryness. The residue was purified by TLC with $CHCl_3$–Me_2CO (9:1). The acetate **20** (66 mg) was extracted from the fraction with *Rf* of 0.26 and another acetate **21** (63 mg) was extracted from that with *Rf* of 0.4. **20**: mp 203—204 °C (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 1755, 1735, 1720, 1240. CD (MeOH) $[\theta]^{25}$ (nm): −2800 (310) (negative maximum). ^{1}H-NMR (FX 100, $CDCl_3$) δ: 1.15 (3H, s, 4-Me and 3H, d, J=7 Hz, 16-Me), 2.04 (3H, s, 3-OAc), 2.13 (3H, s, 6-OAc), 2.34 (1H, d, J=5 Hz, 5-H), 2.51 (1H, d, J=7 Hz, 16-H), 3.69 (2H, s, 19-H_2), 3.80, 4.08 (each 1H, ABq, J=12 Hz, 20-H_2), 4.82 (1H, dd, J=10, 5 Hz, 3-H), 6.37 (1H, d, J=5 Hz, 6-H). *Anal.* Calcd for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19. Found: C, 64.17; H, 7.34. **21**: mp 204—204.5 °C (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 1765, 1735, 1720, 1640, 1220, 1030. ^{1}H-NMR (FX 100, $CDCl_3$) δ: 1.13 (3H, d, J=7 Hz, 16-Me), 1.24 (3H, s, 4-Me), 2.04 (3H, s, OAc), 2.54 (1H, d, J=7 Hz, 16-H), 3.98, 4.20 (each 1H, ABq, J=12 Hz, 20-H_2), 4.06 (2H, s, 19-H_2), 4.74 (1H, dd, J=9, 6 Hz, 3-H), 6.43 (1H, s, 6-H). *Anal.* Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.02; H, 7.37.

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11) The signal of the proton at C-6 in the enmein-type hemiacetal would be a singlet because the dihedral angle between H-5 and H-6 is nearly 90°.
12) These data have been deposited with the Cambridge Crystallographic Data Center.