Terpenoids. LII. The Structures of Trichorabdal F, Trichorabdal G Acetate, and Trichorabdal H. A Comment on the Structure of Shikodonin[1)]

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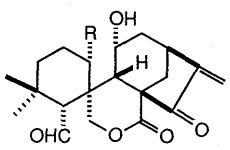
The structures of trichorabdals F and H, and trichorabdal G acetate isolated from *Rabdosia trichocarpa* were determined. It is proposed from the results of structure elucidation of trichorabdal F that the structure of shikodonin should be revised.

Keywords kaurene-type diterpenoid; trichorabdal F; trichorabdal G; trichorabdal H; shikodonin; antitumor activity

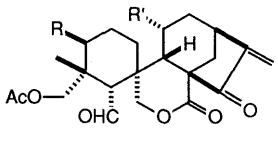
We have reported the isolation of new antitumor diterpenoids, trichorabdals A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), from *Rabdosia trichocarpa*.[2-5)] Further investigation of the crude extracts of this plant led to isolation of trichorabdals F and G as their acetates **6** and **7**, respectively. Trichorabdal H (**8**) was isolated from the same plant collected in Nagano prefecture. Here, we present a full account of the structure determinations of trichorabdal F, trichorabdal H, and trichorabdal G acetate and make a comment concerning the reported structure of shikodonin.

Trichorabdal F acetate (**6**), mp 221—223 °C (dec.), crystallizes from ethyl acetate. On refluxing in tetrahydrofuran (THF) with HCl followed by acetylation, trichorabdal B (**2**) afforded the monoacetate **6** identical with trichorabdal F acetate, along with the corresponding diacetate **9**. This confirmed the structure **6** (*ent*-6α-acetoxy-11β-hydroxy-7,15-dioxo-6,19 : 7,20-diepoxy-6,7-seco-16-kaurene) for trichorabdal F acetate. The acetate **6** regenerated trichorabdal F (**10**) on acidic hydrolysis, indicating that no skeletal rearrangement occurred on acetylation or on hydrolysis.

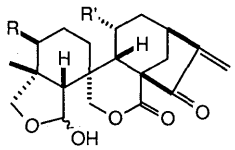
The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of trichorabdal F (**10**) shows a pair of signals for almost every resolvable proton, because trichorabdal F (**10**) exists as an equilibrium mixture **11a** (*ent*-6β,11β-dihydroxy-7,15-dioxo-6,19 : 7,20-diepoxy-6,7-seco-16-kaurene) and **11b** (*ent*-6α,11β-dihydroxy-7,15-dioxo-6,19 : 7,20-diepoxy-6,7-seco-16-kaurene) in solution. The ratio of **11a** and **11b** varied depending upon the solvent and the temperature. The structure **11b** is the same as that proposed for shikodonin[6)] isolated from *Rabdosia shikokiana* in 1978. The structure **11b** for shikodonin was proposed on the basis of an X-ray crystallographic analysis of the corresponding methyl acetal **12**. Since an X-ray analysis of trichorabdal F acetate (**6**) has been performed,[7)] the structure **10** for trichorabdal F is unequivocal. Thus, *O*-methylshikodonin (**12**) should be the methyl acetal of trichorabdal F (**10**). This automatically leads to the conclusion that shikodonin and trichorabdal F have been same structure. However, neither **11a** nor **11b** in the equilibrium mixture gave identical ^{1}H-NMR signals with those reported for shikodonin, as summarized in Table I. Although shikodonin yields *O*-methylshikodonin (**12**) on crystallization from methanol, this is not the case for trichorabdal F. It is clear that the structure of shikodonin should be reinvestigated. The most plausible structure for shikodonin is **15** with α-H at C-5. Because of the strained *trans*-fused 5-membered ring, the cyclic hemiacetal in **15** can easily open to give the corresponding aldehyde **16**, which may undergo epimerization at C-5 *via* **17** in methanol to give the *O*-methylacetal **12** as a final product after recrystallization (Chart 1).



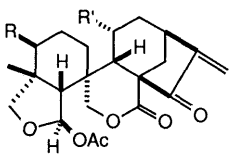
1: R = H
8: R = OAc



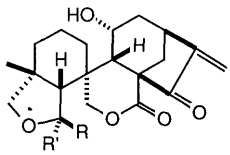
2: R = H, R' = OH
3: R = OH, R' = H
4: R = R' = OH



5: R = OAc, R' = OH
10: R = H, R' = OH
13: R = OAc, R' = H



6: R = H, R' = OH
7: R = OAc, R' = H
9: R = H, R' = OAc



11a: R = H, R' = OH
11b: R = OH, R' = H
12: R = OMe, R' = H

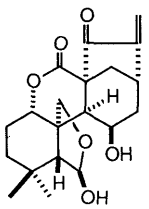


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TABLE I. Pertinent ^{1}H-NMR Data for Trichorabdal F and Shikodonin[a)] in C_5D_5N

	6-H	11-H	18-H_3	19-H_2	20-H_2
Trichorabdal F	6.11 (d, J=5)		0.96	3.43, 3.85 (ABq, J=8)	4.40 (dd, J=13, 2) 5.29 (d, J=13)
		4.52 (m)			
	5.90 (d, J=6)		1.12	3.71, 3.98 (ABq, J=8)	4.86 (dd, J=13, 2) 5.36 (d, J=13)
Shikodonin	4.85 (d, J=3)	3.08 (dd, J=15, 6)	1.15	4.06, 4.24 (ABq, J=12)	3.50 (d, J=8.5) 3.70 (d, J=8.5)

a) Taken from reference 6. J value is in Hz.

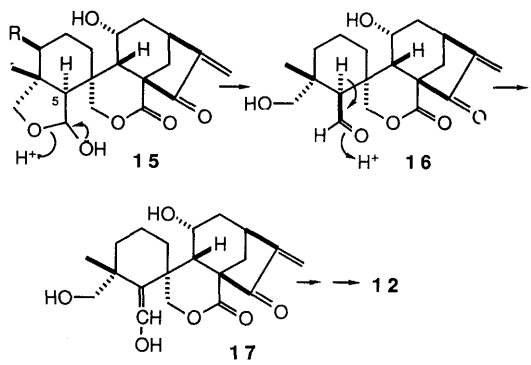

Chart 1

In the preceding paper,[1] we reported that trichorabdal C (**3**) furnished a diacetate on treatment with acetic acid at 90 °C under nitrogen. Trichorabdal G acetate (*ent*-3α,6α-diacetoxy-7,15-dioxo-6,19 : 7,20-diepoxy-6,7-seco-16-kaurene) (**7**) was shown to be identical with the acetate. Thus, the structure **13** is the most probable structure of trichorabdal G.

Close inspection of the spectral data (see Experimental) for trichorabdal H indicates the structure **8** for this diterpenoid. Conversion of trichorabdal H (**8**) into nodosin (**14**)[8] on treatment with 5% hydrochloric acid–acetic acid at 90 °C confirmed the structure **8** (*ent*-1β-atoxy-11β-hydroxy-6,7,15-trioxo-7,20-epoxy-6,7-seco-16-kaurene) for trichorabdal H.

Trichorabdal F acetate (**6**) and G acetate (**7**) have potent *in vivo* antitumor activity against Ehrlich ascites carcinoma inoculated into mice, and this will be the subject of the following paper.

Experimental

Melting points, optical rotations, and spectral data were taken as previously reported.[1]

Isolation of Trichorabdal F Acetate (6) and G Acetate (7) The mother liquor of trichorabdal E described in the experimental section of the preceding paper was evaporated to dryness to give a residue (835 mg), which was dissolved in Ac_2O–C_5H_5N (1 : 1, 2 ml). The solution was left overnight at room temperature. The reaction mixture was evaporated *in vacuo* followed by extractive workup with AcOEt to afford a mixture of acetates (600 mg), which was purified by silica gel column chromatography with $CHCl_3$–acetone (23 : 2) to give the acetates **6** (234 mg) and **7** (140 mg).

Trichorabdal F Acetate (**6**): mp 221—223 °C (from MeOH), $[\alpha]_D^{25}$ −78° (c=0.05, EtOH). UV λ_{max}^{EtOH} nm (ε): 228 (9400). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1755, 1735, 1715, 1640, 1230. ^{1}H-NMR (FX 100, C_5D_5N) δ: 1.01 (3H, s, 4-Me), 2.20 (3H, s, 6-OAc), 2.64 (1H, d, J=5.5 Hz, 5-H), 3.12 (1H, dd, J=8, 4 Hz, 13-H), 3.49, 3.85 (each 1H, ABq, J=8.5 Hz, 19-H_2), 3.63 (1H, d, J=11.5 Hz, 14α-H), 4.08 (1H, dd, J=12, 1.5 Hz, 20-H_a), 4.48 (1H, m, 11-H), 5.32 (1H, d, J=12 Hz, 20-H_b), 5.46, 6.13 (each 1H, s, 17-H_2), 6.85 (1H, d, J=5.5 Hz, 6-H), 6.95 (1H, d, J=4 Hz, OH, disappeared with D_2O). High MS Calcd for $C_{22}H_{28}O_7$: 404.183. Found: 404.181.

Trichorabdal G Acetate (**7**): mp 214—215 °C (from MeOH), $[\alpha]_D^{25}$ −65.2° (c=0.05, EtOH). UV λ_{max}^{EtOH} nm (ε): 230 (7700). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1730, 1720, 1640, 1240. ^{1}H-NMR (FX 100, C_5D_5N) δ: 1.17 (3H, s, 4-Me), 2.03, 2.18 (each 3H, s, OAc×2), 2.40 (1H, d, J=12 Hz, 14α-H), 2.62 (1H, dd, J=5, 2 Hz, 5-H), 2.92 (1H, dd, J=9, 5 Hz, 13-H), 3.69, 3.78 (each 1H, ABq, J=8 Hz, 19-H_2), 3.90 (1H, dd, J=12, 2 Hz, 20-H_a), 4.24 (1H, d, J=12 Hz, 20-H_b), 5.13 (1H, dd, J=12, 4 Hz, 3-H), 5.42, 6.09 (each 1H, s, 17-H_2), 6.80 (1H, d, J=5 Hz, 6-H). *Anal.* Calcd for $C_{24}H_{30}O_8$: C, 64.56, H; 6.77. Found: C, 64.24; H, 6.89.

Isolation of Trichorabdal H (8) The crushed dry leaves (22 g) of *Rabdosia trichocarpa* HARA collected in Nagano prefecture were extracted with MeOH and treated in the same way as described in the preceding paper. The AcOEt extract (1 g) was chromatographed on a silica gel column with $CHCl_3$–Me_2CO (4 : 1) and the residue (106 mg) of the fraction No. 11—16 was separated by preparative thin layer chromatography (TLC) ($CHCl_3$: Me_2CO=9 : 1) to give trichorabdal H (**8**) (24 mg), mp 216.5—219 °C (from AcOEt), $[\alpha]_D^{25}$ +19.4° (c=0.05, MeOH). UV λ_{max}^{MeOH} nm (ε): 229 (8850). IR ν_{max}^{KBr} cm^{-1}: 3420, 2850, 2740, 1740, 1710, 1700, 1635, 1240. ^{1}H-NMR (FX 100, C_5D_5N, 40 °C) δ: 0.86, 1.04 (each 3H, s, 4-Me_2), 2.10 (3H, s, OAc), 2.80 (1H, d, J=4.5 Hz, 5-H), 3.11 (1H, dd, J=9.5, 5 Hz, 13-H), 3.37 (1H, br d, J=12 Hz, 14α-H), 4.46 (1H, br m, 11-H), 4.90, 5.36 (each 1H, ABq, J=12 Hz, 20-H_2), 5.36, 5.98 (each 1H, s, 17-H_2), 5.70 (1H, br t, J=8 Hz, 1-H), 6.79 (1H, br m, OH), 9.95 (1H, d, J=4.5 Hz, 6-H). ^{13}C-NMR (FX 100, C_5D_5N, 50 °C) δ: 204.3 (d, C-6), 201.8 (s, C-15), 170.7, 169.9 (each s, C-7 and $-O\underline{C}OCH_3$), 149.1 (s, C-16), 118.5 (t, C-17), 79.7 (d, C-1), 68.2 (t, C-20), 63.7 (d, C-11), 61.9 (d, C-5), 59.2 (s, C-8), 47.8 (d, C-9), 44.9 (s, C-10), 40.4 (s, C-4), 42.2 (t, C-12), 34.1 (d, C-13), 33.8 (q, C-18), 32.8 (t, C-3), 30.0 (t, C-14), 23.8 (t, C-2), 24.1 (q, C-19), 21.4 (q, $-OCO\underline{C}H_3$). High MS Calcd for $C_{22}H_{28}O_7$: 404.184. Found: 404.184.

Reversion of Trichorabdal F Acetate (6) to Trichorabdal F (10) The acetate **6** (14 mg) was dissolved in THF (5 ml) containing a few drops of 5% HCl, and stirred overnight at room temperature. Extractive work-up with AcOEt followed by purification by column chromatography gave trichorabdal F (**10**) (6 mg), mp 227—230 °C (dec.) (from AcOEt). IR ν_{max}^{KBr} cm^{-1}: 3450, 1738, 1720, 1688, 1650, 1270. ^{1}H-NMR: see Table I.

Trichorabdal F Acetate (6) from Trichorabdal B (2) Trihorabdal B (**2**) (204 mg) was dissolved in THF (5 ml) containing 10 drops of 5% HCl. The reaction mixture was refluxed for 4 h under an N_2 atmosphere, then poured into ice-water and extracted with AcOEt. Usual work-up of the extract gave the residue (185 mg), which was separated by preparative TLC ($CHCl_3$: Me_2CO=2 : 1) to afford trichorabdal F (67 mg). Recrystallization from AcOEt provided pure trichorabdal F (51 mg), which was acetylated with Ac_2O–pyridine to afford a mixture of acetates. Preparative TLC with $CHCl_3$–acetone (4 : 1) furnished trichorabdal F acetate (**6**) (48 mg) and diacetate **9** (1 mg), amorphous, ^{1}H-NMR (FX 100, C_5D_5N) δ: 0.97 (3H, s, 4-Me), 2.13, 2.17 (each 3H, s, OAc×2), 2.88 (1H, d, J=12 Hz, 14α-H), 3.16 (1H, dd, J=8, 4 Hz, 13-H), 3.48 (1H, d, J=8 Hz, 19-H_a), 3.84 (2H, m, 19-H_b and 20-H_a), 4.45 (1H, d, J=12 Hz, 20-H_b), 5.28 (1H, t, J=4 Hz, 11-H), 5.60, 6.18 (each 1H, s, 17-H_2), 6.35 (1H, d, J=5 Hz, 6-H). High MS Calcd for $C_{24}H_{30}O_8$: 446.194. Found: 446.190.

Conversion of Trichorabdal H (8) to Nodosin (14) Trichorabdal H (**8**) (8.3 mg) was dissolved in 5% HCl (1 ml) with 2 drops of AcOH and heated at 90 °C for 4 h under an N_2 atmosphere. When the material (**8**) disappeared on TLC, the reaction mixture was poured into ice-water and extracted with $CHCl_3$. Purification by preparative TLC ($CHCl_3$: Me_2CO=4 : 1) afforded nodosin (**14**) (1.3 mg).

References and Notes

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