Terpenoids. LIII.[1] Antitumor Activity of Trichorabdals and Related Compounds

Kaoru FUJI,*,[a] Manabu NODE,[a] Midori SAI,[b] Eiichi FUJITA,[c] Setsuo TAKEDA[d] and Norio UNEMI[d]

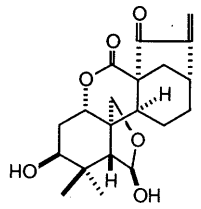
Institute for Chemical Research, Kyoto University,[a] Uji, Kyoto 611, Japan, Faculty of Pharmaceutical Sciences, Setsunan University,[b] Hirakata, Osaka 571-01, Japan, Osaka University of Pharmaceutical Sciences,[c] Matsubara 580, Osaka, Japan and Research Laboratory, Taiho Pharmaceutical Co., Ltd.,[d] Kawauchi-cho, Tokushima 771-01, Japan. *Received October 13, 1988*

Among the three types, enmein-, oridonin-, and trichorabdal-type, of diterpenoids from *Rabdosia trichocarpa*, the latter showed the highest antitumor activity against Ehrlich ascites carcinoma in mice. Their potent activities were attributed to synergistic increase arising from the presence of two active sites in one molecule. *In vitro* activity against HeLa cells and *in vivo* activity against P 388 lymphocytic leukemia of diterpenoids and related compounds were also determined, but no synergistic increase in activity due to plural active sites was observed in those cases.

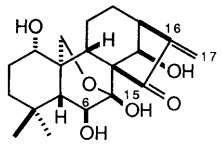
Keywords antitumor activity; synergism; diterpenoid; trichorabdal A; trichorabdal B; trichorabdal C; oridonin; *Rabdosia trichocarpa*

A number of diterpenoids isolated from the genus *Rabdosia* (Labiatae) possess notable cytotoxicity against HeLa cells,[2] KB cells,[3-6] mammary cancer FM 3A/B cells,[7,8] and Ehrlich carcinoma cells.[9] *In vivo* antitumor activity of *Rabdosia* diterpenoids against Ehrlich ascites carcinoma,[2,10,11] Walker intramuscular carcinosarcoma,[5] and P 388 lymphocytic leukemia[12,13] has also been well-established. *Rabdosia trichocarpa* contains two major diterpenoids, enmein (**1**) and oridonin (**2**), both of which have been claimed to have potent cytotoxicity against KB cells.[3] Cytotoxic activity against HeLa cells was also reported for enmein (**1**).[2] These two diterpenoids showed potent *in vivo* activity against Ehrlich ascites carcinoma inoculated into mice.[2,10,11] Preliminary results of a clinical trial with oridonin (**2**) were reported.[14]

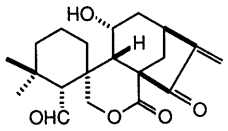
Recently, we isolated new diterpenoids, trichorabdals, from *R. trichocarpa*[15-18] and reported, in a preliminary form, *in vivo* antitumor activity of these diterpenoids and the related compounds against the ascites form of Ehrlich carcinoma and a synergistic increase in activity due to the presence of plural active sites in the molecule.[19] Here we present a full account of our investigation on the antitumor activity of these compounds.



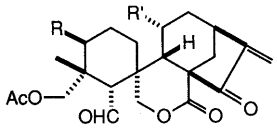
1 (enmein)



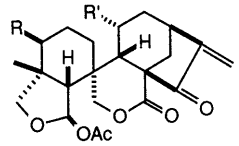
2 (oridonin)



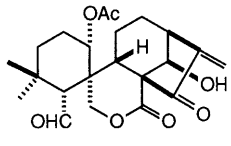
3 (trichorabdal A)



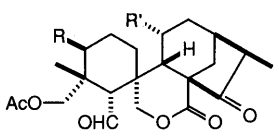
4: R = H, R' = OH (trichorabdal B)
5: R = OH, R' = H (trichorabdal C)
6: R = R' = OH (trichorabdal D)



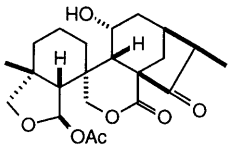
8: R = H, R' = OH
(trichorabdal F acetate)
9: R = OH, R' = H
(trichorabdal G acetate)



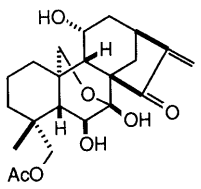
7 (B-secolasiokaurin)



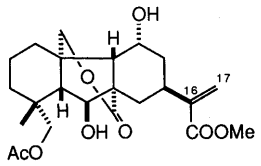
10: R = H, R' = OH
(dihydrotrichorabdal B)
11: R = OH, R' = H
(dihydrotrichorabdal C)



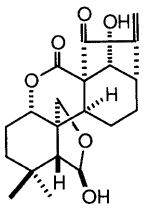
12 (dihydrotrichorabdal F acetate)



13 (longikaurin D)



14
15 (16,17-dihydro)



16 (6,7-secooridonin)

Experimental

Melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-FX 100 spectrometer with tetramethylsilane as an internal standard. High-resolution mass spectrum was obtained with a JEOL JMS-01SG double focusing mass spectrometer.

Materials Isolation of trichorabdals A (**3**), B (**4**), C (**5**) and D (**6**), trichorabdal F acetate (**8**), trichorabdal G acetate (9), and longikaurin D (**12**) has been reported in the preceding papers. Dihydrotrichorabdal B (**10**) and compound **14** were prepared from trichorabdal B (**4**) as described in the experimental section of part LI in this series.[16] Preparation of dihydrotrichorabdal C (**11**) was described in the same section.

B-Secolasiokaurin (7) A solution of lasiokaurin[20] (300 mg) in methanol (60 ml) was treated with $HIO_4 \cdot 2H_2O$ (10 eq) in water (4 ml) and the

mixture was stirred for 26 h at room temperature. After addition of saturated sodium bicarbonate solution (4 ml), the reaction mixture was extracted with dichloromethane. The extract was washed with brine and evaporated, and the residue was recrystallized from ether and dichloromethane to afford B-secolasiokaurin (**7**) (116 mg), mp 164—166.5 °C (from ether–methanol). IR ν_{max}^{KBr} cm^{-1}: 3420, 2720, 1740, 1700, 1690, 1650, 1270, 1230. ^{1}H-NMR: 1.12, 1.19 (each 3H, s), 2.09 (3H, s), 2.25 (1H, d, J=4.4 Hz), 3.05 (1H, br d, J=8 Hz), 4.40 (1H, br s), 5.71 (1H, s, disappeared with D_2O), 4.78 (1H, dd, J=12, 2 Hz), 5.20 (1H, d, J=12 Hz), 5.63 and 6.24 (each 1H, br s), 9.76 (1H, d, J=4.4 Hz). *Anal.* Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.40; H, 7.10.

Compound 15 To a solution of dihydrotrichorabdal B (**10**) (38 mg) in methanol (5 mg) was added 0.15% aqueous sodium hydroxide solution (5 ml) under ice-cooling. After being stirred for 5 min, the mixture was poured into ice-water, acidified with 5% hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give a residue. Purification by column chromatography over silica gel with chloroform–acetone (17:3) as an eluent afforded compound **15** (13 mg), mp 205—207 °C (from chloroform–methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3400, 1735, 1725, 1265. ^{1}H-NMR: 1.17 (3H, d, J=7 Hz), 1.43 (3H, s), 1.88 (1H, d, J=7 Hz), 2.00 (3H, s), 2.48 (1H, m), 3.76 (1H, dd, J=14, 4 Hz), 3.00 (1H, m), 3.54 (3H, s), 4.32, 4.56 (1H, each, ABq, J=12 Hz), 4.50 (2H, m), 4.76 (1H, d, J=7 Hz), 5.08 (1H, d, J=12 Hz). High MS: Calcd for $C_{23}H_{34}O_8$ (M^+): 438.225. Found: 438.226.

Assay of *in Vitro* Activity Using HeLa Cells HeLa cells were cultured as monolayers in Eagle's minimal essential medium (Flow Lab. Inc., U.S.A.) supplemented with 10% calf serum (Flow Lab. Inc., U.S.A.) at 37 °C in a CO_2-incubator. HeLa cells at the stage of logarithmic growth were recovered by trypsinization, washed, and suspended in multi-dish culture trays (Nunc Co., Denmark) at 3×10^3 cells/ml on day 0. Compounds were dissolved in dimethyl sulfoxide (DMSO) and added to each well on day 1 to give final concentrations of 10, 1.0, 0.1, and 0.01 μg/ml. The amount of DMSO was adjusted to give a final concentration of 0.1% in all cases, including the control cultures. After cultivation for 7 d under the conditions described above, HeLa cells were recovered by trypsinization and the number of cells was counted under a microscope. In every experiment, the number of cells in the control under these conditions was usually roughly a hundred times as many as that on day 1. The concentration of a compound required to inhibit cellular growth by 50% (IC_{50}, μg/ml) was obtained by comparing the mean number of cells of three treated groups with that of the control groups.

Test for Antitumor Activity against the Ascites Form of Ehrlich Carcinoma Groups of seven 5-week-old male ddY mice with a body weight of 25—29 g were inoculated intraperitoneally (i.p.) with 2×10^6 Ehrlich carcinoma cells on day 0. A given amount of the test compound in the form of a suspension in saline–ethanol (4:1) was administered i.p. once a day from day 1 to day 7 at a volume of 0.1 ml per 10 g mouse body weight. Antitumor effects were evaluated in terms of the increase in life span.

Test for Antitumor Activity against P 388 Lymphocytic Leukemia Groups of seven 8-week-old male BDF1 mice were inoculated intraperitoneally (i.p.) with 1×10^6 P 388 lymphocytic leukemia cells on day 0. A given amount of the test compound dissolved or suspended in saline–ethanol (4:1) was administerd i.p. once a day from day 1 to day 7 at a volume of 0.1 ml per 10 g mouse body weight. Antitumor effects were evaluated in terms of the increase in life span.

Acute Toxicity Groups of three 5-week-old mice were starved for 12 h and given various doses of the compound suspended in saline. The number of deaths was counted during 3 weeks' observation.

Results

Growth-inhibitory effects of the new diterpenoids and their derivatives as well as oridonin (**2**) on HeLa cells in culture are summarized in Table I. Trichorabdals A (**3**), B (**4**), C (**5**), trichorabdal F acetate (**8**), and trichorabdal G acetate (**9**) showed comparable activity to that of oridonin (**2**). Inactivity of trichorabdal D (**6**) may be ascribed to the insolubility of **6** in the solvent system used.

In vivo activities against Ehrlich ascites carcinoma and P 388 lymphocytic leukemia in mice are given in Tables II and III, respectively. Oridonin (**2**) or trichorabdal B (**4**) was always included in each series of experiments in Tables II and III to evaluate their relative activities.

Table I. Growth-Inhibiting Effect on HeLa Cells in Culture

Compound	IC_{50} (μg/ml)
Oridonin (**2**)	0.50
Trichorabdal A (**3**)	0.19
Trichorabdal B (**4**)	0.47
Dihydrotirchorabdal B (**10**)	>10
Trichorabdal C (**5**)	0.50
Dihydrotrichorabdal C (**11**)	>10
Trichorabdal D (**6**)	>10
Trichorabdal F acetate (**8**)	0.60
Dihydrotrichorabdal F acetate (**12**)	>10
Trichorabdal G acetate (**9**)	0.48
Compound **14**	>10
Compound **15**	>10

Several points are noteworthy in Table II. Trichorabdals A (**3**), B (**4**), and C (**5**) were more potent than oridonin (**2**), which had been claimed to be the most potent[11] (series I and III). Effective doses of trichorabdals B (**4**) and C (**5**) are much lower than LD_{50} (Table IV). Trichorabdal F acetate (**8**) and G acetate (**9**) have activities comparable to those of trichorabdals (series II and V). The activity of compound **14** remains even after the hydrogenation (entries 21 and 22). There is a general tendency that the activity of the compounds possessing a spiro ring is higher than that of enmein- or oridonin-type compounds. A similar tendency is observed in the case of activity against P 388 lymphocytic leukemia, though each activity is rather low (Table III).

Discussion

***In Vivo* Activity against Ehrlich Ascites Carcinoma in Mice** The reaction of an α,β-unsaturated carbonyl system with biologically important sulfhydryl groups plays an important role in the mechanisms by which a compound containing such a system exerts biological activities including antitumor activity.[21] The α-methylene cyclopentanone moiety in oridonin (**2**) is responsible for the antitumor activity because saturation of the *exo*-double bond eliminates the activity. An increase in electrophilicity at C-17 toward sulfhydryl groups by hydrogen bonding between the hydroxyl group at C-6 and the carbonyl group including C-15 was considered to be a crucial factor for the enhanced activity of oridonin (**2**).[11] Trichorabdals showed stronger activity than oridonin (**2**), though they have no hydroxyl group(s) at the proper position for hydrogen bonding with the carbonyl group including C-15. The proton chemical shifts of protons at C-17 and the carbon-13 chemical shift of C-17 (Table V) both indicate that C-17 in oridonin (**2**) is more electron-deficient than those of trichorabdals. This means that the strong activity of trichorabdals cannot be ascribed merely to the electrophilicity at C-17 but must also be due to another factor.

Dihydrotrichorabdal B (**10**), in which the α-methylene cyclopentanone moiety is destroyed, still has a moderate activity (entry 15). Dihydrotrichorabdal F acetate (**12**) also possesses a weak activity though it is no longer act as a Michael acceptor because of the saturation at the double bond between C(16) and C(17). These facts indicate the presence of another active site in these molecules. Oxidative cleavage of the C(6)–C(7) bond in longikaurin D (**13**) (oridonin-type) with periodate afforded trichorabdal B

TABLE II. Antitumor Activity of Trichorabdals and Related Compounds against Ehrlich Ascites Carcinoma in Mice (i.p.–i.p.)

Series	Entry	Compound	Dose (mg/kg/d)	Body weight change (g) (day 8 − day 0)	Survival time (d) mean ± S.D.	ILS (%)	30-day survivors
I		Control		+4.8	10.5±1.9		
	1	Oridonin (**2**)	20	−1.7	17.1±8.4[a]	>63	1/7
			15	+1.6	18.0±6.3[b]	>71	1/7
			10	+2.4	13.6±4.8	29	
			5	+3.6	10.1±2.1	−3	
	2	Trichorabdal B (**4**)	20	−1.2	22.9±8.0[c]	>117	2/7
			15	+0.7	21.7±6.6[c]	>107	1/7
			10	+3.6	15.9±4.1[b]	51	
			5	+5.0	9.9±1.9	−6	
		Control		+3.6	11.7±2.9		
	3	Trichorabdal A (**3**)	20	+0.1	28.3±4.5[c]	>141	6/7
			10	+0.4	27.4±4.4[c]	>134	5/7
			5	+1.1	14.3±4.4	22	
	4	Trichorabdal B (**4**)	20	0.0	28.9±3.0[c]	>146	6/7
			10	+0.7	28.0±5.3[c]	>139	6/7
			5	+3.6	17.0±6.2[a]	>45	1/7
	5	Trichorabdal C (**5**)	20	+0.6	30.0±0.0[c]	>156	7/7
			10	+0.6	27.6±6.0[c]	>135	5/7
			5	+4.3	23.0±7.2[c]	>96	3/7
	6	Dihydrotrichorabdal C (**11**)	20	+4.0	10.9±3.1	−7	
			10	+4.9	12.1±3.3	4	
			5	+3.0	13.1±2.9	12	
	7	Trichorabdal G acetate (**9**)	20	+1.1	29.6±1.1[c]	>152	6/7
			10	+1.4	20.0±7.1[b]	>71	2/7
			5	+2.2	15.1±7.1	29	
III		Control		+8.1	8.1±1.3		
	8	Oridonin (**2**)	20	+1.9	>16.4±7.0	>103[b]	1/7
			10	+4.9	>20.9±9.0	>157[b]	2/7
			5	+6.4	14.1±3.2	74[c]	
	9	Trichorabdal B (**4**)	20	+2.1	>23.1±8.1	>185[c]	3/7
			10	+3.0	16.0±6.4	97[b]	
			5	+7.3	10.4±1.9	29[a]	
	10	Trichorabdal C (**5**)	20	+1.6	21.9±4.7	169[c]	
			10	+5.9	>18.3±5.9	>125[c]	1/7
			5	+8.3	13.4±2.8	66[c]	
	11	Trichorabdal D (**6**)	20	−0.1	8.4±1.7	4	
			10	+6.3	8.4±2.3	4	
			5	+7.1	8.7±1.4	7	
	12	Trichorabdal B (**4**)[d]	80	+6.7	11.3±4.4	39	
			40	+7.6	10.0±1.8	23[a]	
			20	+7.1	8.1±1.8	0	
			10	+8.1	8.9±2.4	9	
	13	Trichorabdal C (**5**)[d]	80	+6.9	11.4±3.7	41[a]	
			40	+2.6	9.0±2.4	11	
			20	+7.7	9.1±1.1	13	
			10	+8.0	10.0±2.0	23[a]	
IV		Control		+4.6	10.0±3.6		
	14	Trichorabdal B (**4**)	20	−1.6	23.1±5.8[c]	>131	1/7
			15	+0.1	24.6±4.3[c]	>146	2/7
			10	+0.9	17.3±3.5[c]	73	
			5	+4.4	12.9±4.4	29	
	15	Dihydrotrichorabdal B (**10**)	20	+3.4	15.1±4.4[a]	51	
			15	+4.6	14.1±5.4	41	
			10	+4.6	11.9±4.0	19	
			5	+5.0	12.1±3.8	21	
	16	Trichorabdal C (**5**)	20	−0.7	25.9±4.2[c]	>159	3/7
			15	+0.3	24.7±4.6[c]	>147	2/7
			10	+0.4	19.9±5.3[c]	99	
			5	+3.0	16.6±4.8[b]	66	
	17	Logikaurin D (**13**)	20	+1.7	14.1±4.3[c]	41	
			15	+3.0	13.3±4.6	33	
			10	+2.6	13.9±4.3	39	
			5	+3.9	9.9±1.6	−1	
V		Control		+2.6	10.0±1.9		
	18	Trichorabdal B (**4**)	20	−1.6	23.3±5.1[a]	>133	2/7
			10	−0.9	20.0±5.4[a]	>100	1/7
			5	+1.6	15.7±3.8[a]	57	
	19	Trichorabdal F acetate (**8**)	20	−1.1	25.7±5.8[a]	>157	4/7
			10	−0.4	23.1±5.4[a]	>131	1/7
			5	+1.3	16.7±3.0[a]	67	
	20	Dihydrotrichorabdal F acetate (**12**)	20	+1.6	14.7±2.1[a]	47	
			10	+2.4	12.1±1.9[c]	21	
			5	+2.3	11.1±1.8	11	
	21	Compound **14**	20	+0.9	15.4±4.0[b]	54	
			10	+1.7	13.7±2.4[b]	37	
			5	+2.0	13.1±3.2[c]	31	
	22	Compound **15**	20	+1.7	15.4±2.7[a]	54	
			10	+1.9	14.7±2.6[a]	47	
			5	+2.1	13.4±2.5[b]	34	
VI		Control		+5.9	11.8±3.5		
	23	Oridonin (**2**)	20	+0.1	19.7±8.8[c]	>67	2/7
			15	+1.4	24.9±6.3[a]	>111	2/7
			10	+4.7	15.9±7.1	35	
			5	+4.7	12.4±3.3	6	
	24	B-secolasiokaunin (**7**)	20	−0.3	21.4±7.9[b]	>82	2/7
			15	+0.6	24.1±5.8[a]	>105	2/7
			10	+3.7	16.6±3.8[c]	41	
			5	+4.9	14.7±4.3	25	

a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$, *d*) o/p.

(**4**)[15,16] bearing a spirolactone and an aldehyde. The antitumor activity of longikaurin D (**13**) was remarkably enhanced by this transformation (see series IV). The same type of structural relationship was found between oridonin (**2**) and B-secolasiokaurin (**7**) (trichorabdal-type). The strong activity of oridonin (**2**) is preserved in B-secolasiokaurin (**7**). It is interesting that the antitumor activity of B-secooridonin (**16**) (enmein-type), obtained from the periodate oxidation of oridonin (**2**), was strikingly reduced.[11] It may be concluded that the compound with the spirolactone ring system, trichorabdal-type, is most active against Ehrlich carcinoma among the three structurally related basic skeletons, oridonin-type, trichorabdal-type, and enmein-type. Although we cannot propose a comprehensive rationalization of the results, the spirolactone aldehyde moiety in the trichorabdal skeleton may function as another active site for the observed activities.[22] Thus, the significant activity of trichorabdal F acetate (entry 19) and trichorabdal G acetate (entry 7) and the moderate activity of dihydrotrichorabdal F acetate (**12**) (entry 20) are attributed to the formation of the spiralactone aldehyde moiety **17** through hydrolysis followed by the opening of the 5-membered hemiacetal ring under physiological conditions (Chart 1). It is interesting that the moderate activity of compound **14** (entry 21), obtained from methanolysis of trichorabdal B (**4**),[15,16] still remains in its dihydro derivative **15** (entry 22). This suggests that the Michael acceptor (an α,β-unsaturated ester group) in **14** is not the active site. Activities of **14** and **15** are again attributed to the latent spirolactone aldehyde grouping in those molecules, because

TABLE III. Antitumor Activity of Trichorabdals and Related Compounds against P 388 Lymphocytic Leukemia in Mice (i.p.–i.p.)

Series	Entry	Compound	Dose (mg/kg/d)	Body weight change (g) (day 8 − day 0)	Survival time (d) mean ± S.D.	ILS (%)	30-day survivors
I		Control		+4.1	10.3 ± 0.8		
	1	Oridonin (**2**)	20	−2.2	10.4 ± 1.7	1	
			15	−0.6	12.3 ± 1.1[a]	19	
			10	+0.3	11.6 ± 1.0[c]	12	
			5	+1.9	11.0 ± 0.6	7	
	2	Trichorabdal B (**4**)	20	−1.7	11.3 ± 1.3	10	
			15	−0.6	12.4 ± 1.0[a]	21	
			10	+0.1	12.1 ± 1.1[b]	18	
			5	+2.6	11.0 ± 0.8	7	
II		Control		+2.4	10.8 ± 0.8		
	3	Trichorabdal B (**4**)	20	−1.7	14.1 ± 2.0[a]	31	
			15	−0.6	13.4 ± 1.7[b]	25	
			10	−0.7	12.7 ± 1.4[b]	18	
			5	+0.3	12.3 ± 1.1[b]	14	
	4	Dihydrotrichorabdal B (**10**)	20	+1.9	11.1 ± 1.1	3	
			15	+2.0	11.1 ± 1.2	3	
			10	+2.3	10.6 ± 0.8	−2	
			5	+2.3	10.4 ± 0.8	−3	
	5	Trichorabdal C (**5**)	20	−1.7	14.1 ± 2.4[b]	31	
			15	−1.1	14.0 ± 2.0[b]	30	
			10	+0.1	12.9 ± 1.4[b]	19	
			5	+0.6	12.1 ± 1.1[c]	13	
	6	Longikaurine D (**13**)	10	+1.9	11.7 ± 1.1	9	
III		Control		+1.3	11.5 ± 1.3		
	7	Oridonin (**2**)	30	−2.6	12.9 ± 1.4	12	
			20	−0.1	13.3 ± 2.3	15	
			10	+0.3	> 16.0 ± 7.1	> 39	1/7
	8	Trichorabdal F acetate (**12**)	30	−1.4	> 20.1 ± 6.8[b]	> 75	1/7
			20	−0.4	16.3 ± 1.7[a]	42	
			10	+1.1	17.0 ± 2.3[a]	48	
	9	Trichorabdal G acetate (**9**)	30	−1.0	13.7 ± 1.3[b]	19	
			20	−0.9	> 15.7 ± 6.7	> 37	1/7
			10	0	> 18.7 ± 5.6[b]	> 63	1/7
	10	Trichorabdal D (**6**)	30	+1.0	11.9 ± 2.7	3	
			20	+0.9	14.3 ± 2.9[c]	25	
			10	+1.9	13.6 ± 1.6[b]	18	
IV		Control		+1.9	10.0 ± 0.8		
	11	Oridonin (**2**)	20	−2.4	11.3 ± 3.9	13	
			15	−2.0	12.9 ± 2.0[b]	29	
			10	−0.4	12.3 ± 1.8[b]	23	
			5	+0.7	11.3 ± 1.8	13	
	12	B-seco-lasiokaurin (**7**)	20	−2.9	12.0 ± 1.9[c]	20	
			15	−3.0	12.7 ± 2.0[b]	27	
			10	−1.8	12.1 ± 1.1[a]	21	
			5	+0.9	11.9 ± 0.7[a]	19	

a) $p<0.001$, *b*) $p<0.01$, *c*) $p<0.05$.

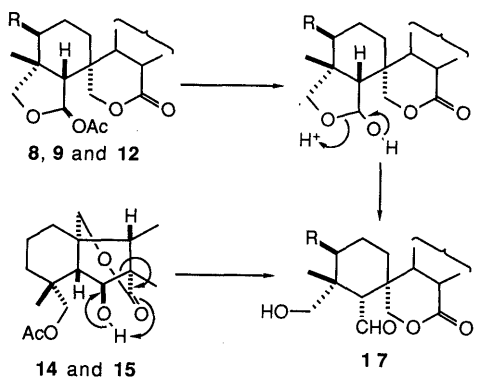


Chart 1

TABLE IV. Acute Toxicity (LD_{50}) of Trichorabdal B (**4**) and Trichorabdal C (**5**) on ddY Male Mice

	Dose (mg/kg)	Mortality in 21 d (i.p.)	(*p.o.*)
Trichorabdal B (**4**)	1000	3/3	0/3
	640	3/3	0/3
	320	3/3	0/3
	160	3/3	0/3
	80	3/3	0/3
	40	0/3	0/3
	20	0/3	0/3
Trichorabdal C (**5**)	1000	3/3	0/3
	640	3/3	0/3
	320	3/3	0/3
	160	3/3	0/3
	80	3/3	0/3
	40	0/3	0/3
	20	0/3	0/3

TABLE V. ^1H- and ^{13}C-Chemical Shifts of 17-H_2 and C-17 of Trichorabdals and Oridonin

Compound	Chemical shift[a] ppm ^1H	ppm ^{13}C
Trichorabdal A (**3**)	5.37, 5.99	117.1
Trichorabdal B (**4**)	5.46, 6.05	117.5
Trichorabdal C (**5**)	5.32, 5.96	117.8
Oridonin (**2**)	5.53, 6.31	119.0

a) Measured in pyridine-d_5.

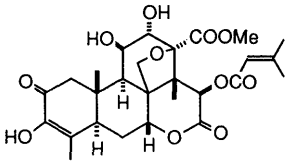
TABLE VI. Effect of Two Active Sites on Antitumor Activity against Ehrlich Ascites Carcinoma

Compound	Active site I	Active site II	ILS (%)
Oridonin (**2**)	Yes	No	63—103
Trichorabdal A (**3**)	Yes	Yes	141
Trichorabdal B (**4**)	Yes	Yes	117—185
Trichorabdal C (**5**)	Yes	Yes	156—169
Trichorabdal D (**6**)	Yes	Yes	4
Dihydrotrichorabdal B (**10**)	No	Yes	51
Dihydrotrichorabdal C (**11**)	No	Yes	−7
Longikaurin D (**13**)	Yes	No	41
Trichorabdal F acetate (**8**)	Yes	Yes	157
Trichorabdal G acetate (**9**)	Yes	Yes	152
Dihydrotrichorabdal F acetate (**12**)	No	Yes	47
6,7-Secooridonin (**16**)	Yes	No	20
Compound **14**	No	Yes	54
Compound **15**	No	Yes	54

Active site I:  . Active site II: –CHO + spirolactone.

a retro-aldol reaction can occur under physiological conditions to afford the structural unit **17** required for the activity (Chart 1). Table VI lists the *in vivo* antitumor activities of diterpenoids and related compounds along with the existence of two or one active sites. With the exception of trichorabdal D (**6**) (entry 11) and dihydrotrichorabdal C (**11**) (entry 6), these results indicate a synergistic increase in antitumor activity of diterpenoids carrying two active sites in the molecule.

***In Vivo* Activity against P 388 Lymphocytic Leukemia in Mice** The general tendency that derivatives of tricho-



18

rabdal-type have stronger activity than those of oridonin-type is maintained except for series IV, though the activity is much lower than that against Ehrlich carcinoma, and the results are not clear-cut. A remarkable increase in antitumor activity against P 388 in mice by connecting two molecules of brusatol (**18**) though an ester linkage was reported by Lee *et al.*[23] This is another example of synergism arising from the presence of plural active sites in a molecule.

Conclusion

We have shown that potent activities of trichorabdals against Ehrlich ascites carcinoma in mice are due to the synergism arising from the presence of two active sites in the molecule. The same tendency was observed for activity against P 388 lymphocytic leukemia *in vivo*, though some uncertainties still remain. However, the results on HeLa cells *in vitro* did not permit us to draw the same conclusion as in the case of Ehrlich ascites carcinoma in mice. Although a comprehensive rationalization of our results remains to be achieved, it is unquestionable that, at least, in certain cases, plural active sites in a molecule results in a synergistic increase in activity against cancer cells.

References and Notes

1) Part LII: M. Node, M. Sai, E. Fujita and K. Fuji, *Chem. Pharm. Bull.*, **37**, 1470 (1989).
2) T. Arai, Y. Koyama, T. Suenaga and T. Morita, *J. Antibiot. Ser. A*, **16**, 132 (1963).
3) M. Yamaguchi, M. Taniguchi, I. Kubo and T. Kubota, *Agric. Biol. Chem.*, **41**, 2475 (1977).
4) I. Kubo, I. Miura, K. Nakanishi, T. Kamikawa, T. Isobe and T. Kubota, *J. Chem. Soc., Chem. Commun.*, **1977**, 55.
5) I. Kubo, I. Miura, T. Kamikawa, T. Isobe and T. Kubota, *Chem. Lett.*, **1977**, 1289.
6) I. Kubo, M. J. Pettei, K. Hirotsu, H. Tsuji and T. Kubota, *J. Am. Chem. Soc.*, **100**, 628 (1978).
7) T. Fujita, Y. Takeda and T. Shingu, *J. Chem. Soc., Chem. Commun.*, **1980**, 205.
8) T. Fujita, Y. Takeda, T. Shingu, M. Kido and Z. Taira, *J. Chem. Soc., Chem. Commun.*, **1982**, 162.
9) S. Han-dong, S. Xi-chang, L. Zhan-wen, Z. Yan-long, Y. Minami, T. Marunaka and T. Fujita, *Chem. Lett.*, **1981**, 753.
10) E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa and H. Kuroda *Experientia*, **32**, 203 (1976).
11) E. Fujita, Y. Nagao, K. Kaneko, S. Nakazawa and H. Kuroda, *Chem. Pharm. Bull.*, **24**, 2118 (1976).
12) Y. Nagao, N. Ito, T. Khono, H. Kuroda and E. Fujita, *Chem. Pharm. Bull.*, **30**, 727 (1982).
13) J. Li, C. Liu, X. An, M. Wang, T. Zhao, S. Yu and G. Zhao, *Yaoxue Xuebao*, **17**, 682 (1982) [*Chem. Abstr.*, **98**, 59801*p* (1983)].
14) H.-D. Sun, Z.-W. Liu, C.-Q. Qin, J.-H. Chao and Q.-Zhao, *Yun-nan Chin Wu Yen Chiu*, **3**, 95 (1981) [*Chem. Abstr.*, **95**, 121032*g* (1981)].
15) E. Fujita, K. Fuji, M. Sai, M. Node, W. H. Watson and V. Zabel, *J. Chem. Soc., Chem. Commun.*, **1981**, 899.
16) K. Fuji, M. Node, M. Sai, E. Fujita, T. Shingu, W. H. Watson, D. A. Grossie and V. Zabel, see Terpenoids Part LI in this issue (accompanying paper).
17) M. Node, M. Sai, E. Fujita and Fuji, *Heterocycles*, **22**, 1701 (1984).
18) M. Node, M. Sai, E. Fujita and K. Fuji, see Terpenoids Part LII in this issue.
19) M. Node, M. Sai, K. Fuji, E. Fujita, S. Takeda and N. Unemi, *Chem. Pharm. Bull.*, **31**, 1433 (1983).
20) E. Fujita and M. Taoka, *Chem. Pharm. Bull.*, **20**, 1752 (1972).
21) S. M. Kupchan, D. C. Fessler, M. A. Eakin and T. J. Giacobbe, *Science*, **168**, 376 (1970).
22) The whole molecular shape of trichorabdal-type molecule could be responsible for the activity. However, our results do not permit us to distinguish between the two possibilities.
23) K.-H. Lee, M. Okano, I. H. Hall, D. A. Brent and B. Soltmann, *J. Pharm. Sci.*, **71**, 338 (1982), and references cited therein.