Communications to the Editor

[Chem. Pharm. Bull. 31(4)1433—1436(1983)]

ANTITUMOR ACTIVITY OF DITERPENOIDS, TRICHORABDALS A, B, AND C, AND THE RELATED COMPOUNDS: SYNERGISM OF TWO ACTIVE SITES

Manabu Node,[a] Midori Sai,[a] Kaoru Fuji,*,[a] Eiichi Fujita,[a]
Setsuo Takeda,[b] and Norio Unemi[b]
Institute for Chemical Research, Kyoto University,[a] Uji, Kyoto 611, Japan and Research Laboratory, Taiho Pharmaceutical Co., Ltd.,[b] Kawauchi-cho, Tokushima 771-01, Japan

Antitumor activity of *Rabdosia* diterpenoids and the related compounds against Ehrlich ascites carcinoma has been studied. In addition to α-methylene cyclopentanone moiety, spirolactone aldehyde grouping functions as an active site in these compounds. Significant increase in activity can be attributed to synergism of two active sites in the molecule.

KEYWORDS ——— diterpenoid; *Rabdosia*; trichorabdal; antitumor activity; synergism

Over 20 diterpenoids of kaurene- and B-secokaurene-type have been isolated from *Rabdosia trichocarpa* Hara. Antitumor activity of some of those diterpenoids has been reported.[1-6] Recently, we have isolated four new diterpenoids, trichorabdals A (1), B (2), C (3), and D (4), (T-A, B, C, and D), along with longikaurin D (5)[7] from the same plant species, and their structures have been elucidated.[8,9] Here we report *in vivo* antitumor activity of these natural products and the related compounds against Ehrlich ascites carcinoma and discuss the structure-activity relationship.

Results are summarized in Table I. T-B (2) is more active than oridonin (6), which has been reported to be the most active diterpenoid from *R. trichocarpa*[2] (Table I, entries 1, 2). T-A (1), B (2), and C (3) possess a high level of acti-

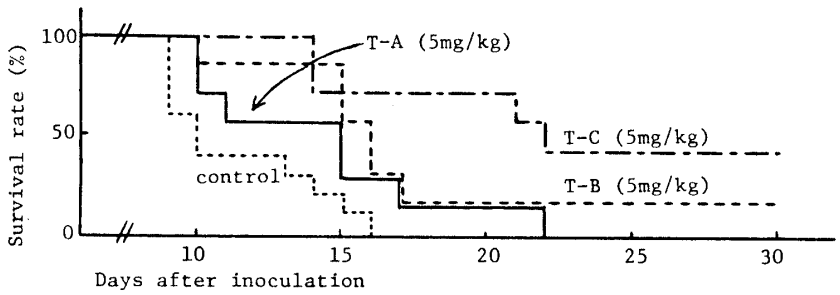
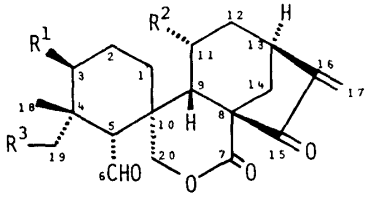


Figure 1. Effect of T-A (1), B (2), and C (3) on Ehrlich Ascites Carcinoma in Mice

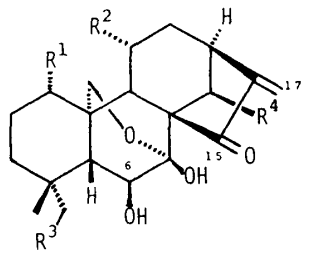
Table I. Antitumor Activity of Trichorabdals and Related Compounds against Ehrlich Ascites Carcinoma

Series	Entry	Compound[a]	Body wt.change (g, day8-day0)	Survival time (day, mean±SD)	ILS (%)	30-day Survivors
I[b]		Control	+ 4.8	10.50±1.90	-	
	1	2	- 1.2	22.86±7.97[c]	>118	2/7
	2	6	- 1.7	17.14±8.36[d]	63	1/7
II[b]		Control	+ 3.60	11.73±2.87	-	
	3	1	+ 0.14	28.29±4.54[c]	>141	6/7
	4	2	0.00	28.86±3.02[c]	>146	6/7
	5	3	+ 0.57	30.00±0.00[c]	>156	7/7
III[e]		Control	+ 4.6	10.00±3.62	-	
	6	2	- 1.6	23.14±5.84[c]	>131	1/7
	7	7	+ 3.4	15.14±4.38[d]	51	3/7
	8	3	- 0.7	25.86±4.22[c]	>159	
	9	5	+ 1.7	14.14±4.26[d]	41	
IV[b]		Control	+ 2.6	10.00±1.94	-	
	10	2	- 1.6	23.29±5.06[c]	>133	2/7
	11	8	- 1.1	25.71±5.82[c]	>157	4/7
	12	9	+ 1.6	14.71±2.06[c]	47	
	13	10	+ 0.9	15.43±4.04[f]	54	
	14	11	+ 1.7	15.43±2.70[c]	54	

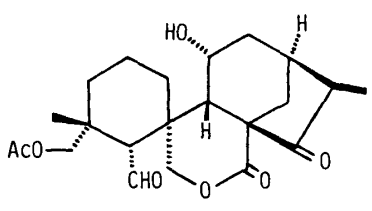
a) Dose: 20 mg/kg/day. b) Five-week-old mice were used. c) $p<0.001$. d) $p<0.05$ e) Six-week-old mice were used. f) $p<0.01$.



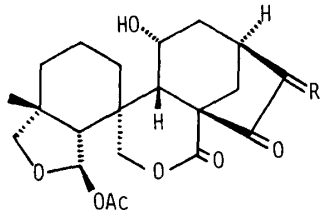
1 : R^1= R^3= H, R^2= OH
2 : R^1= H, R^2= OH, R^3= OAc
3 : R^1= OH, R^2= H, R^3= OAc
4 : R^1=^{2}R = OH, R^3= OAc



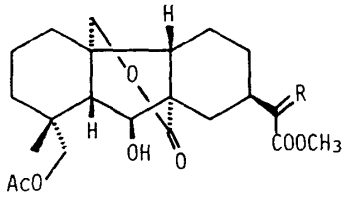
5 : R^1= R^4= H, R^2= OH, R^3= OAc
6 : R^1= R^4= OH, R^2= R^3= H



7



8 : R = CH_2
9 : R = α-H, β-CH_3



10 : R = CH_2
11 : R = H,CH_3

vity (Table I, entries 3-5), in which T-C (3) is more potent than the others (Figure 1). It is a well-accepted view that 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 in question exerts biological activities including antitumor activity.[10] α-Methylene cyclopentanone moiety in oridonin (6) was proved to be the active center for the antitumor activity, because saturation of the exocyclic double bond eliminated its activity. The enhanced activity of oridonin (6) compared with other diterpenoids of kaurene type was ascribed to an increase in electrophilicity at C-17 toward sulfhydryl group due to hydrogen bonding between a hydroxy group on C-6 and a carbonyl group including C-15.[2] However, ^{1}H chemical shifts of protons at C-17 and ^{13}C chemical shifts of C-17 in trichorabdals and oridonin (6) (Table II) indicate that C-17 in the latter is more electron deficient than that in the former, which is inconsistent with the stronger antitumor activity of trichorabdals.

Table II. ^{1}H and ^{13}C Chemical Shifts of 17-H_2 and C-17 in Trichorabdals and oridonin

Compound	Chemical shift,[a] ppm ^{1}H		^{13}C
T-A (1)	5.37	5.99	117.1
T-B (2)	5.46	6.05	117.5
T-C (3)	5.32	5.96	117.8
Oridonin (6)	5.53	6.31	119.0

a) Measured in pyridine-d_5.

Dihydrotrichorabdal-B (7) and dihydro derivative 9, in which α-methylene cyclopetanone system is destroyed, still possess a moderate activity (Table I, entries 7, 12). This strongly indicates the existence of another active center in addition to α,β-unsaturated carbonyl group in these molecules. Periodate oxidation of longikaurin D (5) (kaurene-type) afforded T-B (2) bearing spirolactone ring,[8] the antitumor activity of which is remarkably increased (Table I, entries 6, 9). It may be concluded that the spirolactone aldehyde moiety in trichorabdals and the related compounds functions as another active site. Thus, the significant activity of 8 (Table I, entry 11) is attributed to the formation of spirolactone aldehyde moiety 12 through hydrolysis followed by opening of 5-membered hemiacetal ring *in vivo* (Chart 1). It is interesting that both 10, obtained from methanolysis of T-B (2),[8] and its dihydro derivative 11 exhibit the same level of activity, which suggests that α,β-unsaturated ester grouping cannot be an active site in these molecules. This activity again attributable to the latent spirolactone aldehyde moiety in those molecules, because a retroaldol reaction may take place in the living body to form the structural unit 12 required for the activity.

Present findings disclosed a synergistic increase in antitumor activity of diterpenoids carrying two active sites in the molecule, as exemplified in the case

of longikaurin D (5) and T-B (2). The remarkable enhancement in antitumor activity by connecting two molecules through ester linkage reported by Lee *et al*[11] can be interpreted in terms of synergism of two active sites in a molecule.

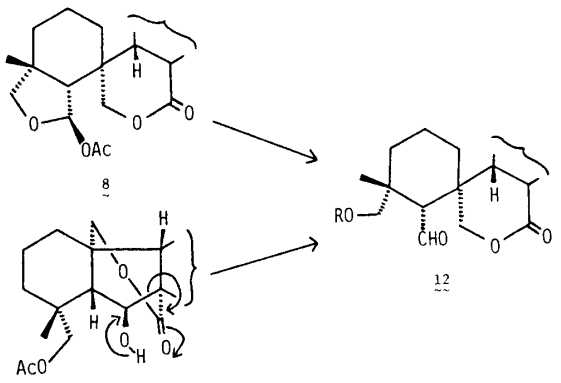


Chart 1

REFERENCES

1) T. Arai, Y. Koyama, T. Suenaga, and T. Morita, J. Antibiot. Ser. A, 16, 132 (1963).
2) E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa, and H. Kuroda, Experientia, 32, 203 (1976).
3) E. Fujita, Y. Nagao, K. Kaneko, S. Nakazawa, and H. Kuroda, Chem. Pharm. Bull., 24, 2118 (1976).
4) M. Yamaguchi, M. Taniguchi, I. Kubo, and T. Kubota, Agric. Biol. Chem., 41, 2475 (1977).
5) E. Fujita, Y. Nagao, T. Kohno, M. Matsuda, and M. Ozaki, Chem. Pharm. Bull., 29, 3208 (1981).
6) Y. Nagao, N. Ito, T. Kohno, H. Kuroda, and E. Fujita, Chem. Pharm. Bull., 30, 727 (1982).
7) T. Fujita, Y. Takeda, and T. Shingu, Heterocycles, 16, 227 (1981).
8) E. Fujita, K. Fuji, M. Sai, M. Node, W. H. Watson, and V. Zabel, J. Chem. Soc. Chem. Commun., 1981, 900.
9) M. Node, M. Sai, K. Fuji, E. Fujita, T. Shingu, W. H. Watson, D. Grossie, Chemistry Lett., 1982, 2023.
10) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, Science, 168, 376 (1970).
11) I. H. Hall, K. H. Lee, M. Okano, D. Sims, T. Ibuka, Y. F. Liou, and Y. Imakura, J. Pharm. Sci., 70, 1147 (1981).

(Received February 26, 1983)