repeated with a pretreatment of carbidopa (120 μM/kg), a peripheral L-amino-acid decarboxylase inhibitor[15,16], administered orally as a suspension in 5% gum acacia, 60 min before the other drug treatments.

The onset of sleep did not vary significantly among the different experiments, but differences were observed in the duration of sleeping time. The effects of various isoquinoline treatments on ethanol-induced narcosis are illustrated in Figure 2. Neither salsolinol (60 μM/kg, 460 μM/kg, 920 μM/kg) nor 3-carboxysalsolinol (7.5 μM/kg, 15 μM/kg, 30 μM/kg, 60 μM/kg) produced any appreciable narcosis when administered alone. Ethanol led to loss of the righting reflex for approximately 1 h (64.3 $\pm$ 3.7 min). When applied in the lowest dose (60 μM/kg) the combination of salsolinol with ethanol produced no prolongation, but at the higher doses (460 μM/kg and 920 μM/kg) sleeping times were significantly prolonged ($p < 0.01$ and $p < 0.005$, respectively). The 3-carboxy analogue (60 μM/kg) significantly protracted the ethanol-induced narcosis ($p < 0.001$). Significant potentiation ($p < 0.025$) was also observed with two lower doses (30 μM/kg and 15 μM/kg) but not with the lowest dose (7.5 μM/kg).

The results demonstrate clearly that 3-carboxysalsolinol is a much more potent potentiator than salsolinol. Because a 30 or a 15 μM/kg dose of 3-carboxysalsolinol produces the same order of potentiation as a 460 μM/kg dose of salsolinol, there appears to be at least a 15-fold difference in potency.

The reason for the much higher potency of the 3-carboxysalsolinol is not definitely known at the present time, but an hypothesis can be offered. It is possible that the amino-acid compound, the 3-carboxy analogue, gains access to the central nervous system (CNS) more readily than the non-carboxylated compound, salsolinol – a relation similar to that of L-DOPA and dopamine[17]. Interaction with ethanol could then occur.

In reviewing the data presented above, it would seem that peripheral formation of salsolinol as a prime mechanism for the potentiation of ethanol sleeping time by dopamine, observed by ourselves[18] and other investigators[3], is unlikely in view of the relatively high doses of salsolinol required. It is noteworthy, however, that salsolinol (460 μM/kg) may still be a more effective potentiator than either DOPET (5.2 mM/kg) or tryptophol (1.5 mM/kg), the reduced metabolites[3,5].

The finding that ethanol sleeping time is potentiated by L-DOPA[4,18] may be the result of peripheral formation of 3-carboxysalsolinol. The high order of potency of the carboxylated compound suggests that the condensation of L-DOPA with acetaldehyde to form 3-carboxysalsolinol may be of importance in potentiating ethanol narcosis when L-DOPA is co-administered.

L-DOPA is known to undergo extensive metabolism in vivo, a factor that has led to the combined use of L-DOPA and peripheral decarboxylase inhibitors in the treatment of Parkinsonian patients[19]. To investigate the possibility that 3-carboxysalsolinol might also be decarboxylated peripherally, further studies which incorporated pretreatment with carbidopa were done. As indicated in Figure 3, carbidopa (120 μM/kg), 3-carboxysalsolinol (7.5 μM/kg) and salsolinol (60 μM/kg) did not cause any loss of righting reflex. In addition, none of these three compounds, in the doses used, enhanced the ethanol-induced narcosis (Figures 2 and 3). The 3-carboxy analogue, however, greatly prolonged ($p < 0.001$) ethanol sleeping time following the carbidopa suppression of peripheral decarboxylase, whereas the result with salsolinol was unchanged. This suggests that peripheral decarboxylation of 3-carboxysalsolinol may occur and that enzyme inhibition permits more of the compound to be available for penetration into the CNS. Upon reaching a central location its action of enhancing the ethanol-induced narcosis may be due to a depressant effect of the 3-carboxy analogue itself or of salsolinol which may be produced by decarboxylation in the CNS.

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Antitumor Activity of the Isodon Diterpenoids: Structural Requirements for the Activity

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Summary. A significant antitumor activity of oridonin (**1**) and lasiokaurin (**2**), the kaurene-type diterpenoids of *Isodon* species, was shown by their i.p. injection to the test mice inoculated by Ehrlich ascites carcinoma. Enmein (**8**), compounds **9** and **3** were also active under larger dose. Subsequently, the relationship between their chemical structure and antitumor activity was investigated, and the activity of oridonin (**1**) and lasiokaurin (**2**) was rationalized in terms of their structural feature.

Isodon japonicus Hara and *I. trichocarpus* Kudo (Labiatae) have been used as a home remedy in Japan. Previously, it was reported that a crude crystalline substance obtained from these plants showed an antitumor activity[2].

Recently, many kaurene and B-seco-kaurene type diterpenoids were isolated from these and other *Isodon* plants, and their structures were clarified[3]. Most of these diterpenoids contain an α-methylene cyclopentanone system in their molecule. We expected that this conjugated system would contribute to the activity, if the diterpenoids are active. Then, the antitumor activity of ori-

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Table I. Antitumor activity of the *Isodon* diterpenoids and the related compounds against Ehrlich ascites carcinoma in mice[h]

Series	Compound	Dose (mg/kg)	Change of body weight (g)	No of survival	MSD[a] (day)	ILS[b] (%)
I	(**1**)[d,e]	10	+ 1.8	4/7	33.8	115 +++[c]
	(**2**)[e]	10	+ 2.1	3/7	34.1	117 +++
	(**4**)[e]	10	+ 5.6	0/7	17.1	9 —
	(**9**)[e]	10	+ 4.9	0/7	16.4	4 —
	(**6**)[e]	10	+ 5.8	0/7	18.3	17 —
	Control	—	+ 6.9	0/8	15.7	—
II	(**5**)[f]	10	+ 7.0	0/8	19.5	7 —
	(**3**)[f]	10	+ 1.6	0/8	20.6	13 —
	(**1**)[f]	5	+ 5.1	0/8	18.9	3 —
		10	+ 1.2	4/8	31.3	71 +
		15	+ 0.2	5/8	33.8	85 ++
		20	− 2.1	Toxic	—	—
	Control	—	+ 7.3	0/8	18.3	—
III	(**1**)[e]	10	+ 1.3	4/7	34.0	95 ++
	(**7**)[e,g]	10	+ 6.6	0/7	18.0	2 —
	Control	—	+ 6.9	0/7	17.7	—
IV	(**8**)[f]	10	+ 1.2	1/8	24.1	39 ±
	Control	—	+ 7.8	0/8	17.3	—
V	(**8**)[f]	25	+ 0.8	2/8	28.0	66 +
	Control	—	+ 6.9	0/8	16.8	—
VI	(**8**)[f]	20	+ 2.5	2/8	27.9	55 +
		40	− 0.6	4/8	33.3	86 ++
	(**1**)[e]	10	+ 2.0	3/7	31.2	74 +
		20	− 1.8	Toxic	—	—
	(**3**)[e]	10	+ 2.8	0/8	23.1	29 ±
		20	+ 3.2	1/8	28.9	61 +
	(**4**)[e]	20	+ 5.7	0/8	19.3	8 —
		40	+ 4.5	0/8	16.8	−6 —
	(**9**)[e]	20	+ 2.7	1/8	19.0	6 —
		40	+ 2.7	3/8	29.6	65 +
	Control	—	+ 7.3	0/8	17.9	—

[a]MSD: means of survival days; [b]ILS: increase of life span; [c]—: ILS < 25%, ±: 25% ≦ ILS < 50%, +: 50% ≦ ILS < 75%, ++: 75% ≦ ILS < 100%, +++: 100% ≦ ILS; [d]LD_{50} value of oridonin: 35 ~ 40 mg/kg; [e]dissolved in water-ethanol (4:1); [f]dissolved in water-ethanol-tween 80 (40:9:1); [g]Testing for higher dose was not carried out, because of its limited availability and less solubility. [h]The male mice of ddY-system (average weight: 20 ± 0.5 g) were used. Ehrlich ascites carcinoma (2×10^6 cells/mouse) was inoculated into peritoneum of the mice.

donin (**1**)[4], lasiokaurin (**2**)[5], enmein (**8**)[6] and the related compounds against Ehrlich ascites carcinoma was investigated by their i.p. injection of 5 ~ 40 mg/kg every 24 h after the tumor inoculation to mice for 7 days, followed by observation for 33 days.

As result (see Table I and Figure 1), oridonin (**1**) and lasiokaurin (**2**) showed a significant activity as expected. Enmein (**8**), compound (**9**)[4] derived from oridonin (**1**), and compound **3**[7] derived from trichokaurin (**6**)[8] were also shown to be active, under a higher dose than that of oridonin (**1**). Both the Δ^{16} saturated derivative **4**[4] and *n*-butane thiol adduct **5**[9], however, did not show any activity. Trichokaurin (**6**), possessing an acetoxy group instead of carbonyl group at the 15 position, does not show the activity. From these facts, the α-methylene cyclopentanone function was established to be the important active center for the activity.

Many antitumor sesquiterpenoids have been knwon possessing an α-methylene γ-lactone function as the active center[10]. But, an antitumor natural product having an α-methylene cyclopentanone system has not been known, except for sarkomycin (**10**)[11,12]. The present diterpenoids discovered by us are regarded as a new group of compounds which belong to this category, but they are characteristic of the kaurene and B-secokaurene type structures with many oxygen functions, when compared with sarkomycin (**10**).

Subsequently, the relationship between activity and structure is described. The hydroxy group at the 1 position has least effect for the activity, because the activities of oridonin (**1**) and lasiokaurin (**2**) (oridonin 1-acetate) are almost the same. An important role of the hydroxy group at the 6 position is seen from the following facts. The activities of enmein (**8**) and compound **9** are $^1/_4$ of that of oridonin (**1**) or less. The presence of a hydrogen bonding between the hydroxy group at the 6 position and the carbonyl group at the 15 position in the oridonin molecule has been shown on the basis of its IR, UV, and

Table II. The C-17 protons chemical shifts of oridonin (**1**), enmein (**8**) and compound **9** in their NMR-spectra[a]

Compounds	Chemical shifts of methylene protons at C-17 (δ_{ppm})	
Oridonin (*1*)	5.53	6.31
Enmein (*8*)	5.43	5.98
Compound *9*	5.40	6.12

[a]Taken in d_5-pyridine.

NMR spectral data[4]. Hence, the carbon atom at the 17 position is expected to be polarized to δ^+ and to increase the reactivity with the nucleophilic agents. In fact, the less electron density at the C-17 atom in oridonin (**1**) as compared with **8** and **9** is supported by the chemical shifts of the methylene protons at C-17 of oridonin in the lower magnetic field than those of **8** and **9** (Table II).

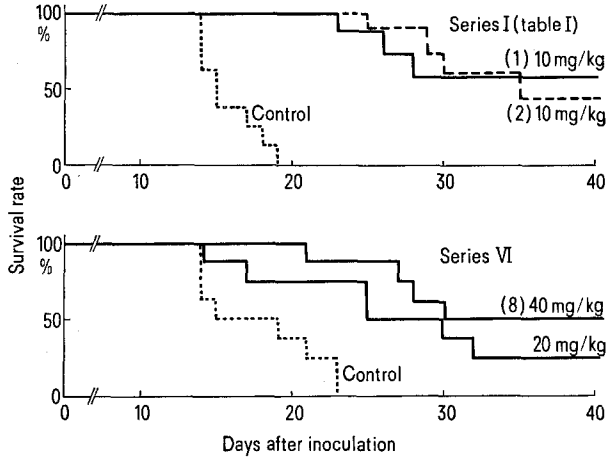

Fig. 1. Effect of oridonin (**1**), lasiokaurin (**2**), and enmein (**8**) on Ehrlich ascites carcinoma in mice.

The activity of 14-deoxyoridonin (**3**) is lower than that of oridonin (**1**), and almost the same as those of **8** and **9**. Thus, the hydroxy group at the 14 position must play an important role in increasing the activity, for instance, as the binding site with a special enzyme in the tumor cells. The hydroxy group at the 7 position is also located very close to the active center and runs parallel with the hydroxy group at the 14 position, hence it may act some role for fixing a nucleophile, cooperating with the hydroxy group at the 14 position. (see Figure 2).

In conclusion, the antitumor activity of oridonin (**1**) and lasiokaurin (**2**) comes from satisfying the following

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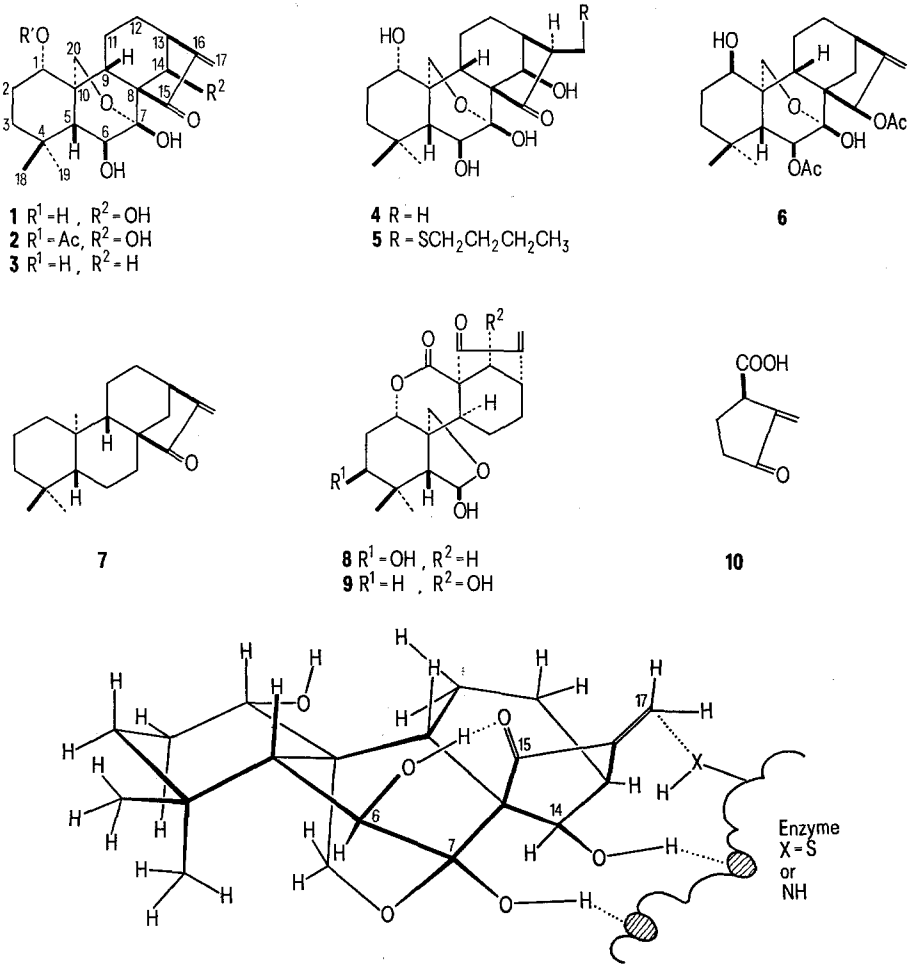

Fig. 2. Hypothetical transition state between or idonin and a specific enzyme in a cancer cell.

necessary conditions: 1. As an active center, an α-methylene cyclopentanone is present in their molecule. 2. Some hydroxy group(s) is present at the position suitable for contact with and binding with an enzyme containing a specific nucleophile. (The β-hydroxy group at the 14 position and/or the hydroxy group at the 7 position.) 3. A hydrogen bonding between the hydroxy group at the 6 position and the carbonyl group at the 15 position is present to enhance the electrophilicity of the carbon atom at the 17 position.

Addendum. After the manuscript was submitted, we learned that ARAI et al.[13] investigated the antitumor activity of enmein, its diacetate, and dihydroenmein, of which only the partial structures had been elucidated, and suggested the activity to be attributed to the exocyclic methylene group attached to 5-membered cyclic ketone on the basis of the results of their biological test. Our findings also support their preliminary experiments and generalize the concept.

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Heart Norepinephrine Concentration after Chronic Alcohol Ingestion in the Rat

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Summary. The effect of long-term alcohol ingestion on the norepinephrine concentration of the heart was investigated in rats. The alcoholic animals showed a highly significant increase in cardiac norepinephrine concentration as compared with the corresponding controls. It is further suggested that continued exposure to high levels of norepinephrine may play a role in the development of cardiomyopathy in chronic alcoholism.

There is considerable evidence of an association between excessive alcohol consumption and heart disease in man[1–7]. The pathogenesis of alcohol-induced cardiac lesions, however, has not been clearly established. JAMES and BEAR[8] have suggested that the chronic cardiac effects of alcohol are mediated through a metabolite, acetaldehyde, and due to chronic depletion of stored norepinephrine. On the other hand, ALEXANDER[9] has advanced the view that the development of alcoholic cardiomyopathy is consequence of an increase in the heart levels of catecholamine and serotonin. In a recent paper, POHORECKY[10] has reported that, following an acute dose of alcohol or 2 weeks of chronic alcohol intake, there is no change in the heart norepinephrine concentration. In the present investigation, a prolonged period of alcohol ingestion was used. The size of the hearts of alcoholic and control rats was studied and the catecholamine concentration determined.

Materials and methods. 39 5-week-old Wistar strain male rats were assigned randomly to 6 treatments, i.e., 6 rats were allowed to drink only alcohol for 4 weeks, 6 rats were fed isocalorically for 4 weeks, 9 rats were allowed to drink only alcohol for 12 weeks, 6 rats were fed isocalorically for 12 weeks, 6 rats were allowed to drink only alcohol for 24 weeks, and 6 rats were fed isocalorically for 24 weeks. A solution of 32% (v/v) ethyl alcohol in 25% (w/v) sucrose in water was given to rats in the three experimental groups[11], while rats in the three control groups were given no alcohol.

After 4 weeks on test, 6 experimental and 6 control rats were sacrificed by dislocation of the neck. Also after 12 and 24 weeks on test, the remaining animals were sacrificed in the same manner. The hearts were rapidly removed, cleaned, and weighed. Heart norepinephrine was separated and assayed by the method of ANTON and SAYRE[12,13]. The catecholamine values were expressed in μg free base/g of wet tissue weight.

Results. The general appearance of the experimental rats was essentially similar to that of the controls. However they weighed 20–25% less than the controls. In animals drinking alcohol, inebriation was a common

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Table I. Effect of chronic alcohol ingestion (4 weeks) on heart norepinephrine concentration and heart: body weight ratio.

	Control ($n = 6$)	Experimental ($n = 6$)	Deviation from control (%)	Statistical significance (p)
Norepinephrine (μg/g)	0.597 ± 0.056	0.611 ± 0.045	0	NS
Heart ratio (g/100 g)	0.363 ± 0.021	0.372 ± 0.042	0	NS

Values are means ± SEM (standard mean error). n = number of animals.