# Recent Progress in the Development of Natural *ent*-Kaurane Diterpenoids with Anti-tumor Activity

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**Abstract:** The *ent*-kaurane diterpenoids are widely distributed in China, some of which have high natural abundance in plants of *Isodon*, *Pteris*, *Gnaphalium*, *Diplospora*, *Croton* and some other species. These compounds exhibit significant anti-tumor, antibacterial and anti-inflammatory activities, which have attracted the attention of medicinal chemists. This review focuses on the recent advances in the research of derivatives, anti-tumor activity, mechanism of action, and structure-activity relationships of *ent*-kaurane diterpenoids. All of these will show the potential in the development of new anti-tumor agents in natural products.

**Keywords:** *ent*-kaurane, diterpenoids, derivatives, anti-tumor activity, mechanism of action, structure-activity relationships.

## INTRODUCTION

Malignant neoplasm or cancer seriously threatens human health and takes the second place among all the causes of mortality, just inferior to the cardio- and cerebro-vascular diseases [1]. Cancer causes about 13% of all human deaths. Only in 2007, 7.6 million people in the world died of cancer [2].

Cancer can be treated by surgery, radiation, biological therapy, hormone therapy, photodynamic therapy (PDT), chemotherapy and a combination of the above [3]. And the anticancer drugs usually used in current clinical treatment have many adverse effects, such as the harm to human immune system, leading to gastrointestinal disorders. In recent years, a wide variety of natural diterpenoids have been reported to exhibit low toxicity and high therapeutic efficacy [4].

Up to now, more than 600 diterpenoids have been identified and investigated in China, some of which have high natural abundance in plants of *Isodon* (*Rabdosia*), *Pteris*, *Gnaphalium*, *Diplospora*, *Croton* and some other species [5]. These diterpenoids can be classified structurally into four groups (Figs. **1-4**): (A) *ent*-kauranes, (B) *ent*-6,7-seco-kauranes, (C) *ent*-8,9-seco-kauranes, (D) the others. Group A is then divided into (a) C-20-non-oxygenated kauranes and (b) C-20-oxygenated kauranes [6]; group B is divided into (a) the enmein types and (b) the spirolactone types, and group D is divided into C-7, C-20-bonded *ent*-kauranes and 1:1 complex of natural diterpenoids [7,8].

So far, a lot of *ent*-kauranoids with a diversity of highly oxygenated structures have been isolated from plants of genus *Isodon* (*Rabdosia*) which are widely distributed in southwestern China. These compounds exhibit significant anti-tumor, antibacterial and anti-inflammatory activities [9]. In order to limit the topics, it is the aim of the present review to only cover *ent*-kaurane diterpenoids with anti-tumor activity.

## 1. DERIVATIVES AND ANTI-TUMOR ACTIVITY OF *ENT*-KAURENE

Oridonin is a widely distributed *ent*-kaurene in the *Rabdosia* plants and exhibits anti-tumor activity for the treatment of esophageal cancer, gastric cancer, liver cancer, lung cancer, nasopharyngeal cancer, colon cancer, bladder cancer, cervical cancer and leukemia.

### 1.1. Structural Modifications of *ent*-Kaurene Type Diterpenoids

In 1981, Fujita group first synthesized a series of 6-*O*-, 14-*O*-acyl oridonin derivatives (**24**, **25**) to study the structure-activity relationships (SAR) of diterpenoids with $\alpha,\beta$-unsaturated ketone moiety. It was found that the 14-*O*-acyl derivatives showed strong cytotoxicity against Ehrlich ascites carcinoma in mice, and the activity increased with the elongation of acyl carbon chain; whereas the 6-*O*-acyl derivatives have no inhibitory activity against Ehrlich ascites carcinoma in mice [34-36].

In 1990, Zhou group first converted oridonin (**5**) into a known natural product eriocalyxin B (**8**) by six-step reactions in a total yield of 11% [37].

In order to study the SAR of oridonin derivatives, Yan synthesized oridonin-6-*O*-$\beta$-D-glucopyranoside (**26**) from oridonin and tetra-*O*-benzoyl-$\alpha$-D-glucopyranosyl bromide *via* protection, Koenigs-Knorr reaction and deprotection in a total yield of 23.0% [38].

In 2006, a series of 1-*O*-monoacyl, 12-*O*-monoacyl, 1,12-*O*-diacyl, and 11,12-dehydrated excisanin A 7,14-acetonides analogues were synthesized by Aoyagi group through structure modification of excisanin A (**4**), and it was found that

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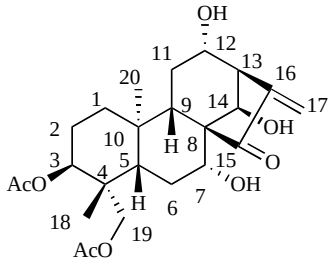
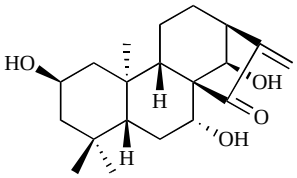
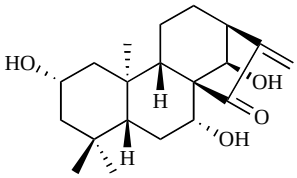
(**a**)



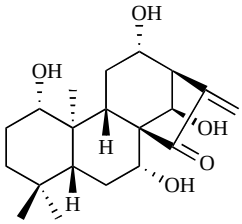
**(1)** pharicinins D [10]



**(2)** umbrosin A [11]

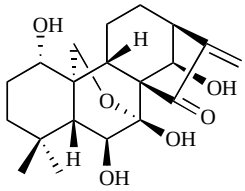


**(3)** mebadonin [12]

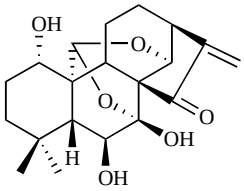


**(4)** excisanin A [13]

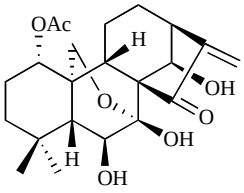
(**b**)



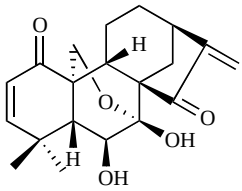
**(5)** oridonin [14,15]



**(6)** ponicidin [16]



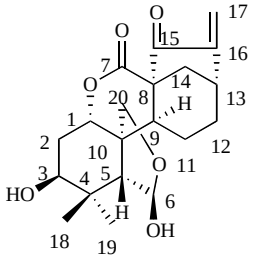
**(7)** excisanin H [17]



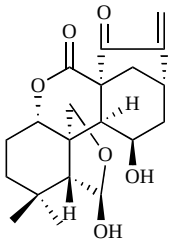
**(8)** eriocalyxin B [18]

**Fig. (1).** Structures of *ent*-kaurane type diterpenoids (A): C-20-non-oxygenated *ent*-kauranes (**a**), and C-20-oxygenated *ent*-kauranes (**b**).

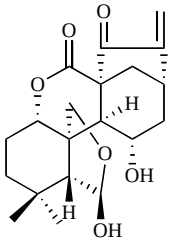
(**a**)



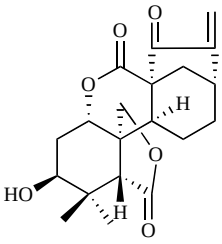
**(9)** enmein [19]



**(10)** nodosin [20,21]

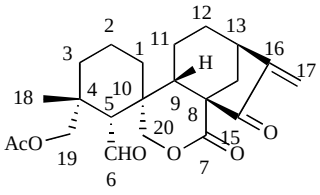


**(11)** epinodosin [22,23]

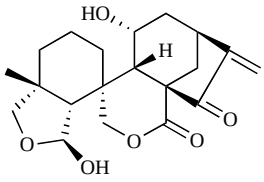


**(12)** ememodin [24]

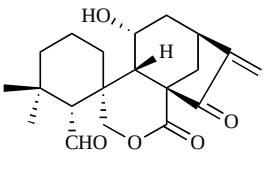
(**b**)



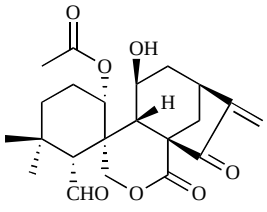
**(13)** effusin [25]



**(14)** shikodonin [26]

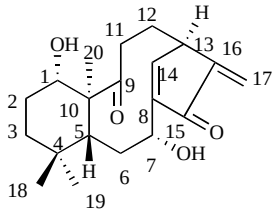


**(15)** trichorabdal A [27]

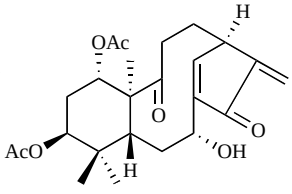


**(16)** taihangexcisoidesin B [28]

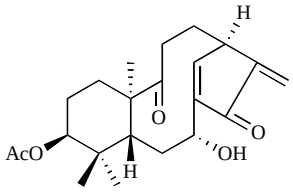
**Fig. (2).** Structures of *ent*-6,7-seco-kaurane type diterpenoids (B): enmein types (**a**), and spirolactone types (**b**).



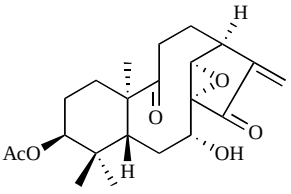
**(17)** rabdolatifolin [12]



**(18)** shikodomedin [29]

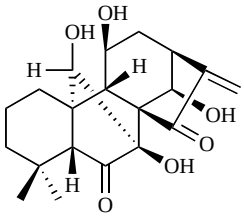


**(19)** shikoccin [30]

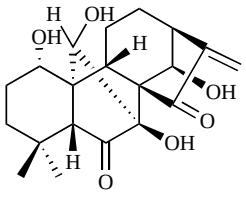


**(20)** epoxyshikoccin [31]

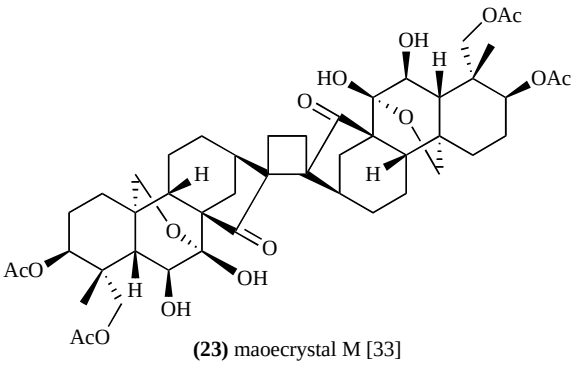
**Fig. (3).** Structures of *ent*-8,9-seco-kaurane type diterpenoids (C).



**(21)** phyllostachysin A [32]

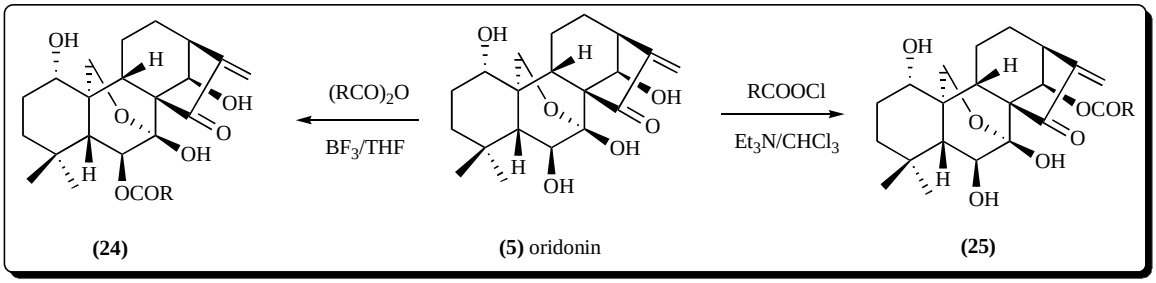


**(22)** rubescensin D [32]

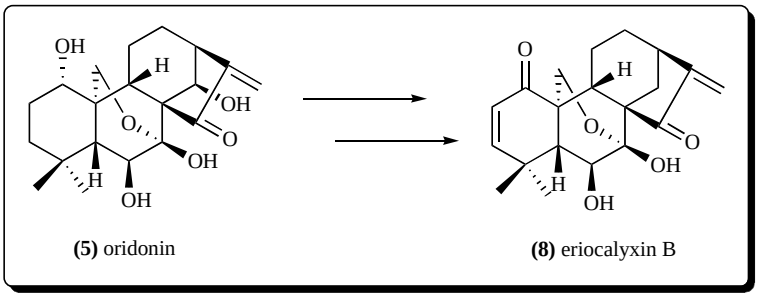


**(23)** maoecrystal M [33]

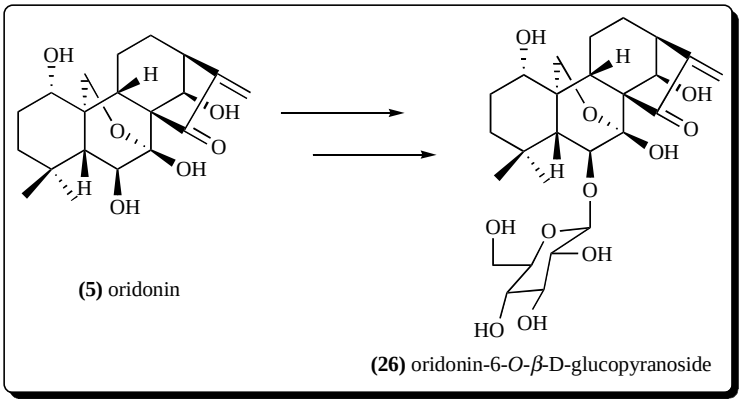
**Fig. (4).** Structures of other type diterpenoids (D): 7,20-bonded *ent*-kaurane types and 1:1 complex of natural diterpenoids.




**(24)** **(5)** oridonin **(25)**



**(5)** oridonin **(8)** eriocalyxin B



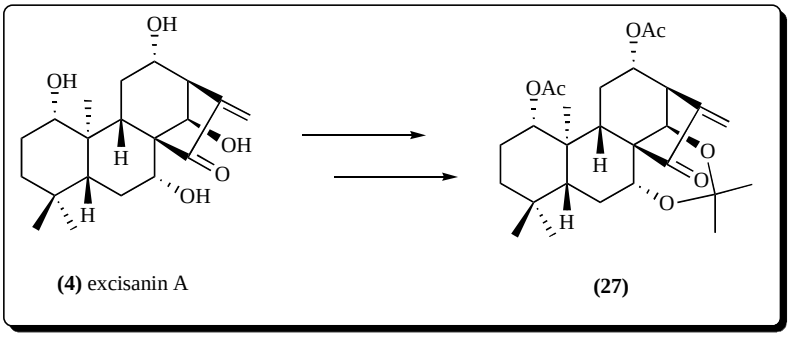
**(5)** oridonin

**(26)** oridonin-6-*O*-*β*-D-glucopyranoside

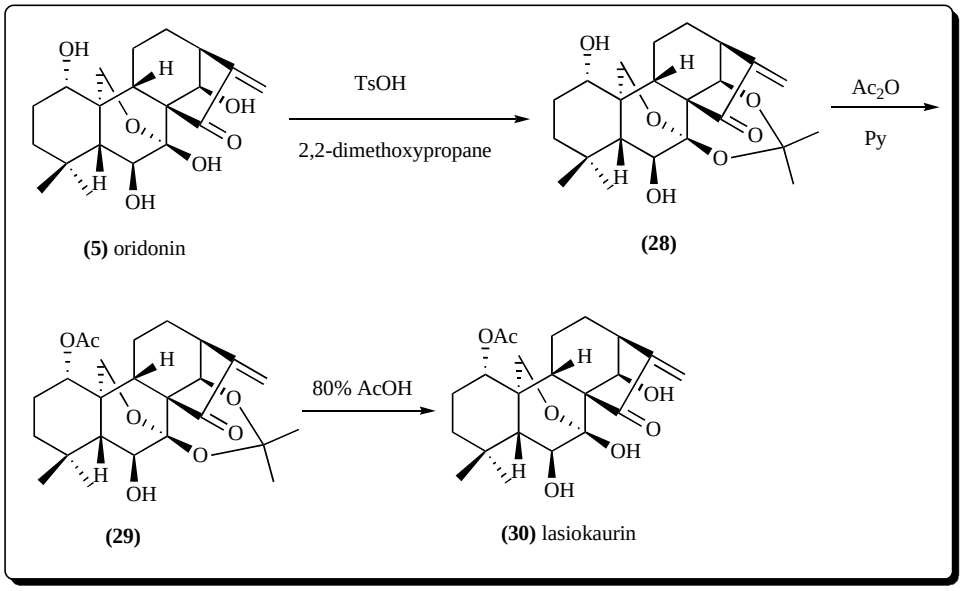
compared with excisanin A, the cytotoxicity of some derivatives against P388 cells has increased nearly by 20 times. Compound 1-,12-*O*-diacetylexcisanin A 7,14-acetonide (**27**) was the most potent with $IC_{50}$ values of 0.18 $\mu$M [39].

In 2006, starting from oridonin, Yan synthesized natural product lasiokaurin (**30**) *via* selective acetonide protection, acetylation and deprotection in 69.7% overall yield. The antiprotozoon activity of lasiokaurin was more potent than oridonin with $IC_{50}$ values of 25 $\mu$M, suggesting that substitution at C-1 of oridonin may be helpful to the enhancement of its anti-tumor activity [40].

In 2007, Amino-derivatives of oridonin (**31-34**) were synthesized starting from oridonin *via* Mannich reaction by Yan group. The derivatives have significant anti-tumor activ-

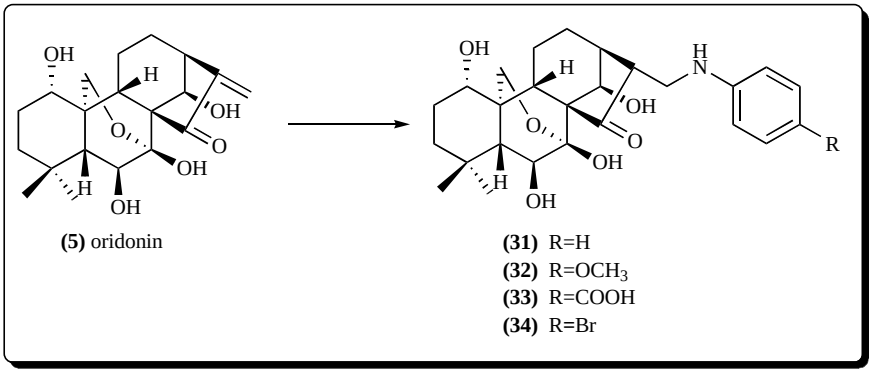



**(4)** excisanin A **(27)**




**(5)** oridonin **(28)**

**(29)** **(30)** lasiokaurin




**(5)** oridonin

**(31)** R=H
**(32)** R=$OCH_3$
**(33)** R=COOH
**(34)** R=Br

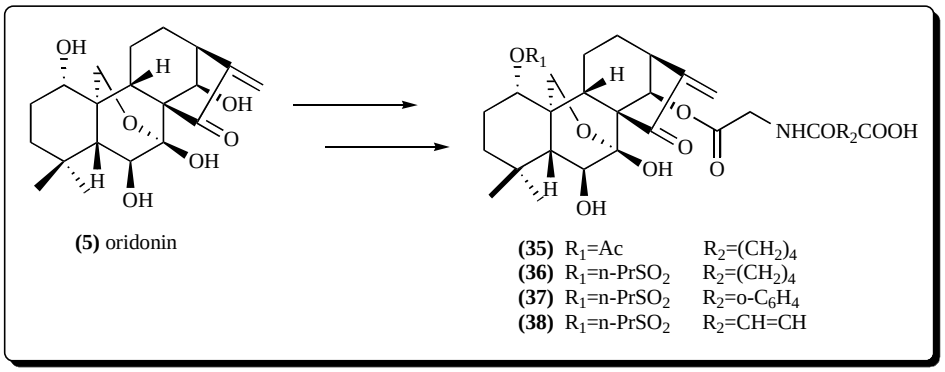
ity, especially against the proliferation of KB cells, with the inhibition more than 90% at the dosage of 20 mg/L [41].

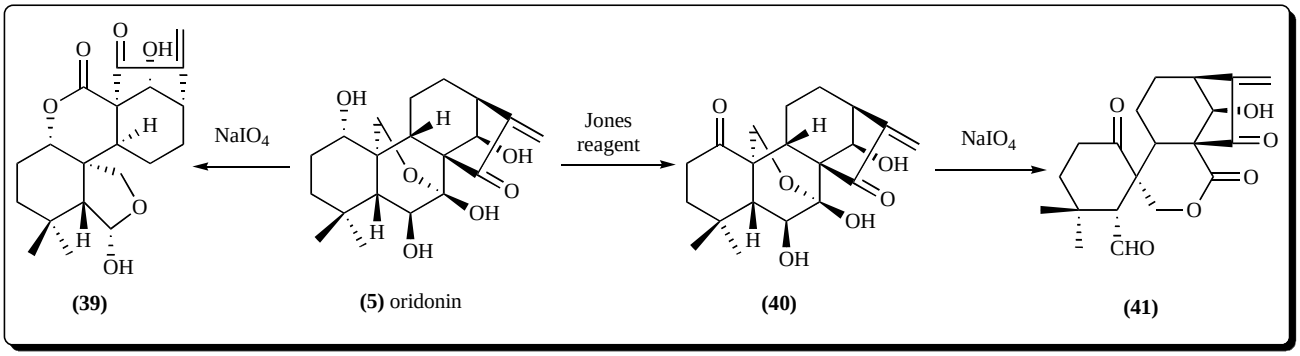
In 2008, our group synthesized some novel 1-*O*- and 14-*O*- oridonin derivatives (**35-38**). All of the derivatives exhibited stronger inhibitory activity against six cancer cell lines (BGC-7901, SW-480, HL-60, Bel-7402, A549, and B16) than oridonin *in vitro*, in which two compounds were the most potent with the $IC_{50}$ values of 0.84 $\mu$M in HL-60 cell and 1.00 $\mu$M in Bel-7402 cell, respectively. And they were more potent than oridonin and cyclophosphamide *in vivo* with the ratio of inhibition 64.9% in mice with H22 [4].

### 1.2. Structural Modifications of *ent*-6,7-seco-Kaurene Type Diterpenoids

The conversion of kaurene-type to 6,7-seco-kaurene-type compounds is easily achieved by periodate oxidation of a 6,7-dihydroxyl-kaurane derivative. The oxidative compounds are ether enmein- or spirolactone-type. When the starting material has a hydroxyl group at C-1, enmein-type compounds **(39)** were obtained; otherwise, spironolactone-types **(41)** would be formed [42].

Eriocalyxin B (**8**) is regarded as a promising *ent*-kaurene for the treatment of cancer because of its potent activity and

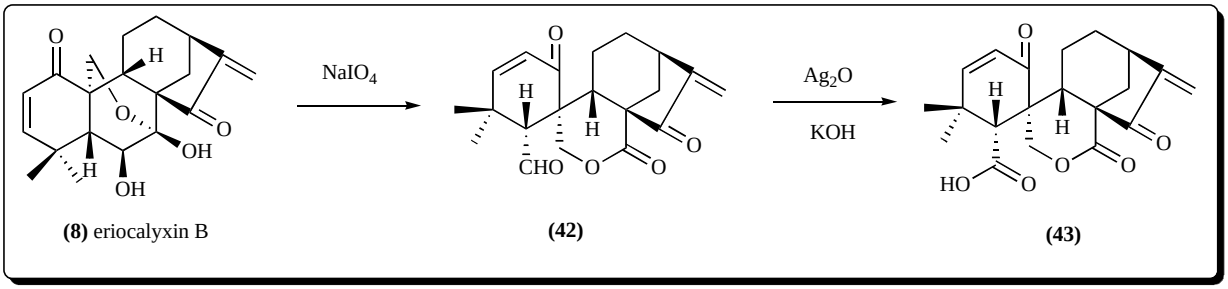


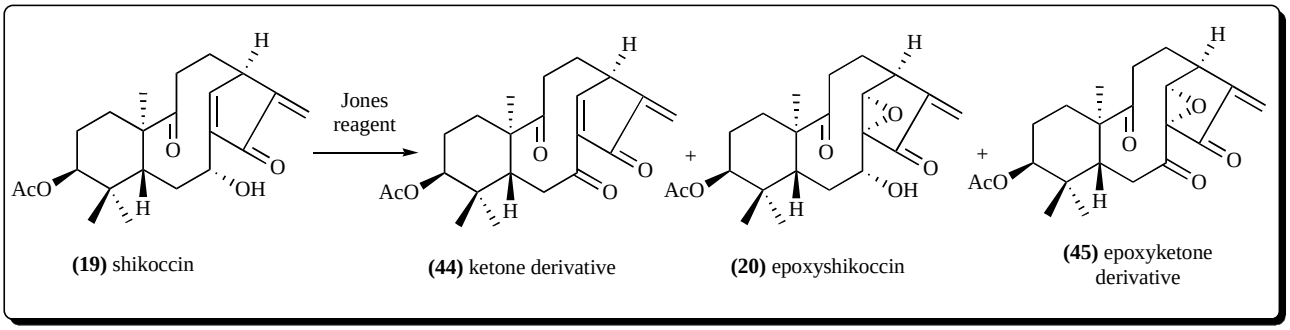



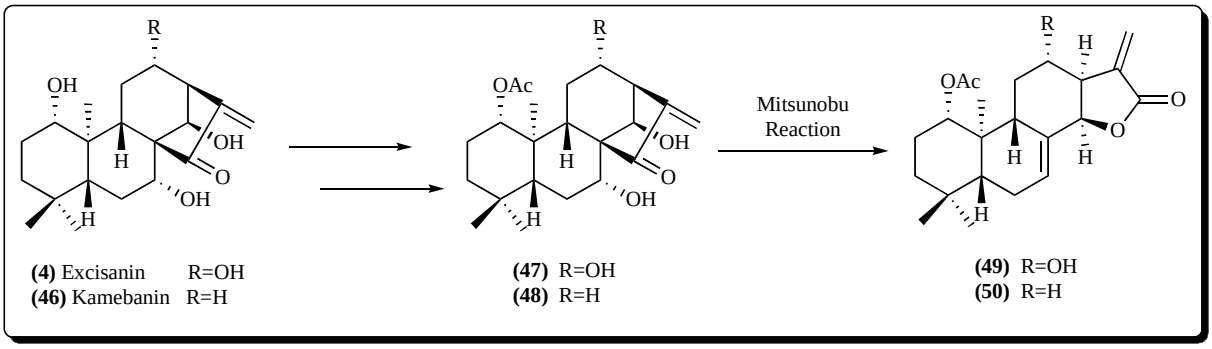

novel mechanism of action. By systematic modification of eriocalyxin B, nineteen derivatives were obtained and their cytotoxicities against five tumor cell lines were evaluated. The results showed that the 6,7-seco-derivative with spirolactone aldehyde moiety (**42**) has good inhibitory activity against the tested cell lines with $IC_{50}$ values less than 1 $\mu$M. Whereas, conversion of aldehyde to carboxylic acid gave the inactive compound **(43)**. SAR studies indicate that $\alpha,\beta$-unsaturated ketone moieties in ring A and D may be the key active sites [6].

### 1.3. Structural Modifications of *ent*-8,9-seco-Kaurene Type Diterpenoids

Fujita *et al*. isolated five new diterpenoids from *Rabdosia shikokiana* (Makino) Hara vas. *Occidentalis* (Murata) Hara (Labiatae) [43]. One of these diterpenoids shikoccin **(19)** was then oxidized, yielding the corresponding ketone derivative **(44)**, epoxyshikoccin **(20)** which was identical with a known natural product, and an epoxyketone derivative **(45)** in 6.4%, 5.3% and 13.2% yields, respectively [44].






**(4)** Excisanin R=OH
**(46)** Kamebanin R=H

**(47)** R=OH
**(48)** R=H

**(49)** R=OH
**(50)** R=H

### 1.4. Structural Modifications of *ent*-8,15-seco-Kaurene Type Diterpenoids

Yutaka synthesized some novel cytotoxic *cis*-fused *α*-methylene *γ*-lactones from 7,14-dihydroxy-*ent*-kaurenes **(4, 46)** under Mitsunobu reaction conditions. The prepared compounds **(49, 50)** showed a moderate inhibitory activity against P388 murine leukemia cells [45].

## 2. STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

Arai first reported the anti-tumor activity of the crude crystalline material isolated from *Rabdosia trichocarpa* [46]. Subsequently, enmein was found to have anti-tumor activity against Ehrlich ascites carcinoma, while dihydroenmein was inactive. It suggests that the *α*-methylene cyclopentanone is essential for activity.

Fujita examined the anti-tumor activity of oridonin, lasiokaurin, enmein, enmein-3-acetate and related compounds against Ehrlich ascites carcinoma in mice, and found that the compounds with the *α*-methylene cyclopentanone moiety possess anti-tumor activity, on the other hand, the thiol adduct and the dihydro derivatives of oridonin were inactive. The facile addition of soft nucleophiles such as alkanethiols and L-cysteine to *α,β*-unsaturated ketone moiety of oridonin or enmein supported the hypothesis that the anti-tumor activity of *Rabdosia* diterpenoids may be attributed to deactivation of SH-enzymes (or SH-coenzymes). The stronger activity of oridonin and lasiokaurin may be ascribed to hydrogen-bonding between the 6-OH and the C-15 carbonyl groups; the hydroxyl groups at C-7 and C-14 were assumed to play a role as binding sites to specific enzymes in the cancer cell lines (Fig. **5**) [47].

The anti-tumor activity of some acylated oridonins has also been examined, and it was found that an increase in the chain length of 14-acyl oridonins tended to increase anti-tumor activity, for example, the 14-dodecanoyl, 14-tetradecanoyl, and 14-hexadecanoyl oridonin were more active than oridonin. On the other hand, acylation of the 6-OH group resulted in deactivation, which supported the important role of this hydroxyl in hydrogen-bonding to the C-15 carbonyl [36]. And the introduction of terminal carboxylic acid moiety to the 14-OH of oridonin appeared crucial for an increase in the cytotoxicity of the target compounds. Furthermore, 1-oxo, 1-propylsulfonyl or 1-acyl oridonin derivatives displayed more potent anti-tumor activity than oridonin, especially 1-acetyl oridonin derivatives showed the best cytotoxicity against cancer cell lines, suggesting that free 1-OH of oridonin is not essential for its anti-tumor activity [4].

In summary, the oridonin derivatives with 1-OH substituted by carbonyl, sulfonyl or acyl have stronger anti-tumor activity than oridonin with original 1-OH. Substitution on 6-OH of oridonin would cause the total loss of anti-tumor activities. 14-OH with both ester side chain of lipophilicity and terminal carboxylic acid moiety appeared crucial for an increase of cytotoxicity, and the cytotoxicity increased with the elongation of the introduced acyl carbon chain. The SAR study with hydroxyl in other position has not been reported. *α,β*-Unsaturated ketone moiety was proved to be the key

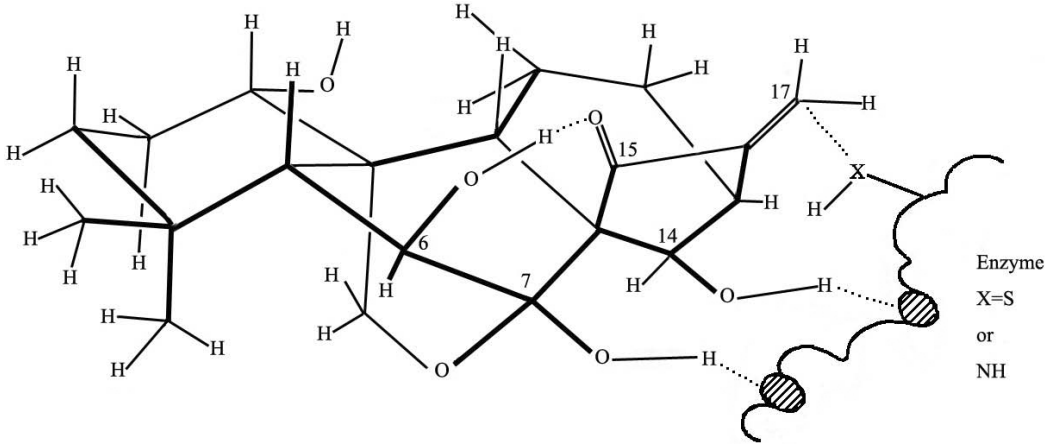



**Fig. (5).** Hypothetical transition state between oridonin and specific enzyme in a cancer cell.

point for cytotoxicity, since normal ketone derivatives were also synthesized, only providing inactive compounds.

Acylation of enmein-type 6,7-seco-oridonin led to an increase in anti-tumor activity, since the 14-*O*-acyl, propionyl, valeryl of enmein had stronger inhibitory activity against six cancer cell lines K562, MCF-7, CaEs-17, Bel-7402, Hela, and A549 than enmein-type 6,7-seco-oridonin [42].

The anti-tumor activity of spirolactone-type diterpenoids has also been studied. The results showed that shikodonin possesses significant cytotoxicity *in vitro* and anti-tumor activity *in vivo* against Ehrlich ascites carcinoma in mice [26]. The anti-tumor activity of trichorabdals A-B (Fig. **2**) and related diterpenoids against Ehrlich ascites carcinoma in mice was more potent than that of oridonin. In contrast to inactive dihydroenmein and its analogues, the trichorabdal dihydro derivatives were still active. Hence, the trichorabdals might possess a second active site, presumably the spirolactone aldehyde site, and the enhanced activity of the trichorabdals might be caused by synergism between the two active sites [48]. Recent examination of biological activity of *ent*-6,7-seco-kaurenes type of 1-oxo-oridonin showed that the spirolactone-type derivatives possessed good activity against K562, A549 and CaEs-17, Bel-7402, Hela, while the enmein type of oridonin derivativies were less active than the spirolactone type. These results revealed that introduction of spirolactone aldehyde moiety could improve the anti-tumor activity [42].

Relatively weak anti-tumor activity was found in the *ent*-8,9-seco-kaurene-type diterpenoids. The oxidative products of shikoccin exhibited greatly enhanced activity compared with that of shikoccin, which is probably attributed to a synergistic effect of the plural active centers [44, 49].

## 3. MECHANISM OF ANTI-TUMOR ACTIVITY

Compared to the research on derivatives and anti-tumor activity, the investigations on mechanism of action of *ent*-kaurane diterpenoids is still lagging.

### 3.1. Ras/Raf/ERK Signal Pathway

Ikejima found that U937 cells showed susceptible to apoptosis induced by 27 $\mu$M oridonin and an agonistic anti-Fas IgM mAb (CH-11) (500 ng/ml) as a Fas-sensitized positive control. Caspase 8 inhibitor z-Ile-Glu-Thr-Asp, but neither caspase 1 inhibitor Ac-Val-Ala-Asp nor caspase 10 inhibitor z-Ala-Glu-Val-Asp effectively blocked oridonin-induced cell death as well as DNA fragmentation. Western blot analysis showed the up-regulated expression of Fas, FasL, and FADD, and down-regulated expression of pro-caspase 8, suggesting that Fas/FasL pathway was activated in oridonin-induced cell apoptosis. Further, stimulation of U937 cells with oridonin and CH-11 resulted in significant ERK MAPK activation. However, inhibition of ERK by PD98059 reversed oridonin-induced cell death as well as the activation of caspase 8, indicating that ERK-mediated control occurred upstream of caspase 8. Simultaneously, ERK activation accounted for the release of cytochrome *c*, but failed to influence decreased Bcl-2 expression induced by oridonin. Taken together, these results suggested that Fas/FasL signaling pathway-mediated ERK activation sensitized U937 cells to mitochondrial pathway-mediated apoptosis induced by oridonin [50].

Li *et al*. investigated signaling events involved in oridonin-induced apoptosis in human epidermoid carcinoma A431 cells. It was found that the total tyrosine kinase activity and the protein expressions of epidermal growth factor receptor (EGFR) were inhibited. Phosphorylated EGFR was decreased in oridonin-induced A431 cell apoptosis. Expression of EGFR downstream effector proteins, Grb2, Ras, Raf-1, and ERK, was also down-regulated by oridonin. Moreover, the oridonin-induced apoptosis was augmented by the Ras inhibitor manumycin A, Raf-1 inhibitor GW5074, or ERK inhibitor PD98059, suggesting that inactivation of Ras, Raf or ERK participates in oridonin-induced apoptosis. Thus, oridonin-induced apoptosis in A431 cells might through blocking EGFR and its downstream Ras/Raf/ERK signal pathway [51].

### 3.2. $G_2$/M Cell Cycle Arrest

Ye found that oridonin inhibited BGC-823 cells growth with $IC_{50}$ of 22 $\mu$M. It induced apoptosis in a dose-dependent manner. In addition, it could decrease mitochondria membrane potential, increase intracellular $Ca^{2+}$, and activate pro-caspase 3. BGC-823 cells were arrested in $G_2$/M cell cycle phase with lower expression of cyclin A protein. The up-regulation of p53 was observed before apoptosis occurrence and cell cycle arrest. It suggests that oridonin inhibits the proliferation of BGC-823 cells through $G_2$/M cell cycle arrest and apoptosis induction, which is mediated by influx of $Ca^{2+}$, up-regulation of p53, activation of caspase-3, and down-regulation of cyclin A [52].

Cheng found that oridonin induces L929 cell $G_2$/M arrest and apoptosis, which is regulated by promoting ERK-p53 apoptotic pathway and suppressing PTK-mediated survival pathway. $G_2$/M phase arrest was associated with down-regulation of cell cycle related cdc2, cdc25c and cyclin B levels, as well as up-regulation of p21 and p-cdc2 levels. It was also found that the interruption of p53 activation decreased oridonin-induced apoptosis, and blocking ERK by specific inhibitors or siRNA suppressed oridonin-induced p53 activation. Moreover, inhibition of PTK, protein kinase C, Ras, Raf or JNK activation increased oridonin-induced apoptosis. Also, the level of Ras, Raf or JNK was down-regulated by oridonin, and the inhibition of PTK, Ras, Raf activation decreased p-JNK level [53].

In 2010, Kang reported that the mechanism involved in oridonin-induced growth inhibition, including apoptosis and $G_2$/M phase arrest, in human laryngeal carcinoma HEp-2 cells deficient in functional p53. Oridonin triggered the mitochondrial apoptotic pathway, as indicated by increased Bax/Bcl-2 ratios, reduction of $\Delta\Psi_m$, and substantial increase in apoptosis-inducing factor and cytochrome *c*. Inhibition of caspase-9 in HEp-2 cells did not protect the cells from oridonin-induced apoptosis, and cleaved caspase-9 was not detected, indicating that apoptosis occurred *via* a caspase-9-independent pathway. The results also suggested that $G_2$/M phase arrest and apoptosis mediated by oridonin occurred *via* a p53-independent but in a p21/WAF oridonin-dependent

manner in HEp-2 cells. In addition, the generation of ROS was found to be a critical mediator in growth inhibition induced by oridonin. The results indicate that oridonin is a potentially effective agent for the treatment of laryngeal squamous cell carcinoma [54].

### 3.3. Akt Pathway

Hu found that oridonin suppressed the proliferation of the Hela cell line in a dose- and time-dependent fashion. Its treatment down-regulated the activation of protein kinase B (Akt), the expression of forkhead box class O (FOXO) transcription factor, and glycogen synthase kinase 3 (GSK3). Oridonin also induced the release of cytochrome c accompanied by the activation of caspase-3 and poly-adenosine diphosphate-ribose polymerase cleavage. In addition, z-Asp-Glu-Val-Asp-fmk, an inhibitor of caspases, prevented caspase-3 activation and abrogated oridonin-induced cell death [55].

Jin group reported that oridonin induced the release of cytochrome *c* accompanied by activation of caspase-9, caspase-3 and cleavage of poly (ADP-ribose) polymerase (PARP). These events were all inhibited by z-Val-Ala-Asp-fmk, a universal inhibitor of caspases. Oridonin treatment dephosphorylated constitutively active AKt, FOXO transcription factor, and glycogen synthase kinase 3. In addition, oridonin decreased the phosphorylation of ERK and increased the phosphorylation of p38 MAPK and JNK. Furthermore, oridonin treatment could down-regulate the expression of the inhibitor of apoptosis protein in osteosarcoma cells. All together, the results suggested that oridonin is able to inactivate Akt and ERK and activate p38 MAPK and JNK signalling pathways in osteosarcoma cells causing the suppression of proliferation and induction of mitochondria- and caspase-dependent apoptosis [56].

### 3.4. Autophagy

Recently, we noticed that there were some investigations on the mechanism of action of oridonin. Liu reported that after treatment with oridonin for 48 h, the percentage of disruption of mitochondrial membrane potential ($\Delta\Psi_m$) gradually increased in a dose-dependent manner along with marked changes of cell apoptosis, and necrotic cells increased remarkably after the cells were treated with oridonin for 72 h; Western blotting showed cleavage of the caspase-3 zymogen protein (32 kDa) with the appearance of its 20 kDa subunit when apoptosis occurred; expression of Bcl-2 and Bcl-XL was down-regulated remarkably while expression of Bax and Bid was up-regulated concurrently after the cells were treated with oridonin for 24 h. Notablely, the expressions of Fas and other Bcl-2 family members including Bak and Bad remained constant before and after apoptosis. It could be concluded that oridonin had significant antiproliferation effects on HPB-ALL cells by induction of apoptosis as well as directly causing cell necrosis and that oridonin-induced apoptosis on HPB-ALL cells was mainly related to the disruption of $\Delta\Psi_m$ and activation of caspase-3 as well as down-regulation of anti-apoptotic protein Bcl-2, Bcl-XL, and up-regulation of pro-apoptotic proteins Bax and Bid [57].

Recent studies have shown that MCF-7 cells undergo autophagy under some conditions, such as tamoxifen treatment and starvation. Cui investigated autophagy in MCF-7 cells under oridonin treatment and further examined the relationship between autophagy and apoptosis. After 3-MA (the specific inhibitor of autophagy) pre-culture, MCF-7 cells were exposed to oridonin, and the growth inhibitory ratio, morphologic changes, DNA fragmentation, proteins expression, autophagic ratio and apoptotic ratio were evaluated. Oridonin inhibited the proliferation of MCF-7 cells and induced autophagy *in vitro*. MDC (a specific dye for autophagosome) recruitment and typical apoptotic features, including apoptotic bodies, membrane blebbing as well as nuclear condensation, were induced by oridonin. Oridonin down-regulated the phosphorylation of ERK, whereas those of JNK and P38 kinase were up-regulated. Under the condition of oridonin treatment, 3-MA significantly reduced the autophagic level, and the apoptotic cell ratio was also declined. Furthermore, combined treatment with oridonin and 3-MA up-regulated ERK phosphorylation and down-regulated JNK and P38 kinases phosphorylation compared with oridonin alone treatment groups, indicating that autophagy facilitated apoptosis in oridonin-induced MCF-7 cells. In addition, 3-MA application down-regulated DNA ladder and Bax expression but up-regulated Bcl-2 expression, compared with oridonin alone treatment. Taken together, oridonin simultaneously induced MCF-7 cells for both apoptosis and autophagy, and in the settings, inhibition of autophagy induced lower apoptotic level, therefore, autophagy participated in up-regulation of apoptosis [58].

Zhang reported that apoptosis and autophagy were simultaneously induced by oridonin time-dependently in HT1080 cells, and inhibition of autophagy by 3-MA decreased oridonin-induced apoptosis, indicating that they acted in synergy to mediate cell death. In addition, treatment with oridonin caused an increase in NF-$\kappa$B and p53 activities in a time-dependent manner. Inhibition of NF-$\kappa$B or p53 activation by its specific inhibitor PDTC or pifithrin-$\alpha$ respectively, significantly reduced both oridonin-induced apoptosis and autophagy accompanied by the decrease in Beclin 1 and LC3 levels. Further experiments revealed that oridonin-induced p53 activation was reduced by the NF-$\kappa$B inhibitor, whereas the activation of NF-$\kappa$B was not affected by p53 inhibition. NF-$\kappa$B promotes oridonin-induced apoptotic and autophagic cell death through regulating p53 activation in HT1080 cells [59].

## 4. PERSPECTIVES OF *ENT*-KAURANE DITERPENOIDS

Nature has been a source of medicinal products for millennia, and during the past century, many useful drugs have been developed from natural sources, particularly plants. Now the researchers all over the world have given more attention on Chinese traditional medicine than before. Meanwhile a series of key scientific research projects have been set up to encourage the research of Chinese herbal in China. With maturing separation and purification methodology, the *ent*-kaurane type diterpenoids can be obtained conveniently and abundantly from the plants widely distributed in China, such as *Isodon*, *Pteris*, *Gnaphalium*, *Diplospora*, *Croton* and some other species. A large number of results proved that this class of compounds has unique anti-tumor mechanism and low toxicity. The development of these compounds

should be of great value and wide application. At present, the commercial availabilities of the *ent*-kaurane type diterpenoids are mainly the mixture of natural plant extract products, such as anticancer pill, rubescens tablet, rubescens tea. So far, no single chemical entity of *ent*-kaurane diterpenoids has been used in the clinical treatment. We believe that in the near future, novel medications prepared from this class of compounds will play an important role in the treatment of cancer.

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