GLYCOSIDES FROM KU-DING-CHA, A KIND OF CHINESE TRADITIONAL TEA

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Introduction

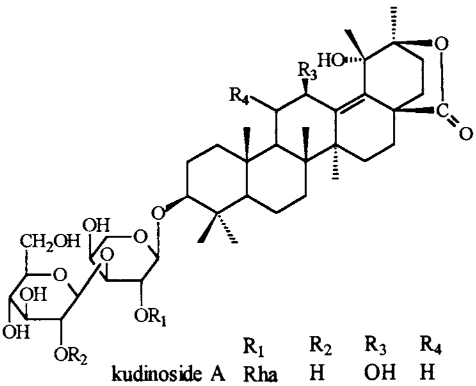
As a kind of special tea, Ku-Ding-Cha has been used in the southern China from many hundred years ago. Instead of tea, local people always drink Ku-Ding-Cha in the summer for clearing away heat and toxic materials in body, and satisfy one's thisrt. It is also used in folk medicine as a diuretic and slimming agent, and for the treatment of hypertension, sore throat as well as inflammation. As for its original materials, there are more than ten plants from different families and genera being used (Table.1) in different places of China. In Yunnan, Sichuan and Guizhou provinces, the leaves of *Ligustrum* (Oleaceae) species are mainly used as Ku-Ding-Cha, while in Guangxi, Guangdong, Hunan and Jiangxi provinces, the leaves of *Ilex* (Aquifoliaceae) species are commonly used[1]. As a part of studies on plant glycosides and Chinese tea plants in our research group, we have paid attention to these interesting plants from several years ago. This is a brief review for our chemical and pharmaceutical works on these plants.

Table 1 The original plants of Ku-Ding-Cha in different areas of China

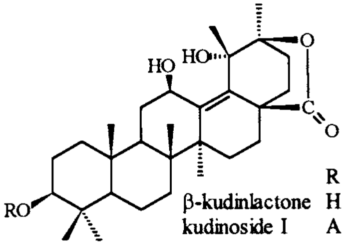
Original plants	Family	Place
Ligustrum pedunculare Rehd.	Oleaceae	Sichuan
L. purpurasceas Y.C.Yang	Oleaceae	Yunnan
L. japonicum var. *pubescens* Koidz.	Oleaceae	Guizhou
L. robustum (Roxb.) Bl.	Oleaceae	Guizhou
Ilex cornuta Lindl. ex Paxt.	Aquifoliaceae	Zhejiang
I. kudincha C.J.Tseng	Aquifoliaceae	Guangxi, Guangdong
I. macrophylla	Aquifoliaceae	Guangdong
I. latifolia Thunb.	Aquifoliaceae	Zhejiang, Hunan
Cratoxylum prunifolium (Kurz) Dyer	Guttiferae	Guangxi
Ehretia thyrsiflora (S.et Z.) Nakai	Boraginaceae	Guangxi
Photinia serrulata Lindl.	Rosaceae	Zhejiang

Glycosides from *I. kudincha*

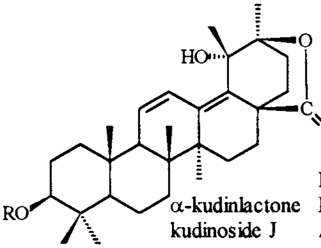
I. kudincha C.J.Tseng is a new species endemically distributed in Guangxi province and commonly used as a material of Ku-Ding-Cha in the locals. After suspended in water, the 70% EtOH extracts of the dried leaves were extracted with $CHCl_3$, EtOAc and n-BuOH, respectively. Further repeatedly separation by column chromatography and HPLC led to the isolation and purification of a lot of triterpenoidal saponins and sapogenins from each fractions. Among them, sixteen new saponins which named as kudinosides A-G, K-Q, S and T, together with zigu-glucoside I were isolated from n-BuOH fraction; two new saponins, kudinosides I and J, were obtained from EtOAc fraction; and from $CHCl_3$ fraction, α- and β-kudinlactones as two new free lactonic aglycones were afforded[3,4]. Kudinosides A-F, I and J are monodesmosides, which aglycones contain a six-member lactone ring between C-20 and C-28. Kudinosides G, K, L-Q, S and T, as well as zigu-glucoside I are bisdesmosides, which have two sugar chains attached at the hydroxyl group of C-3 positions and C-28 carboxyl group of the aglycones, respectively.



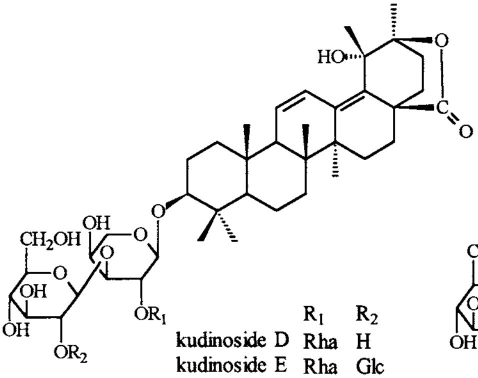
	R1	R2	R3	R4
kudinoside A	Rha	H	OH	H
kudinoside B	H	Glc	OH	H
kudinoside C	Rha	Glc	OH	H
kudinoside F	Rha	H	H	OH



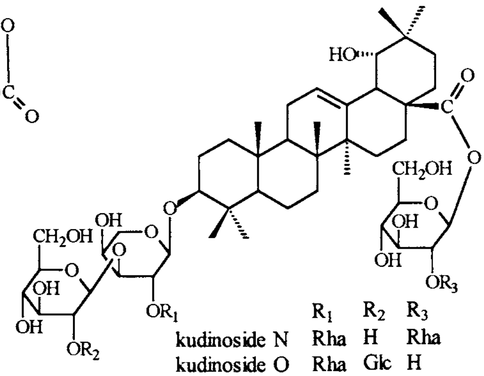
	R
β-kudinlactone	H
kudinoside I	Ara



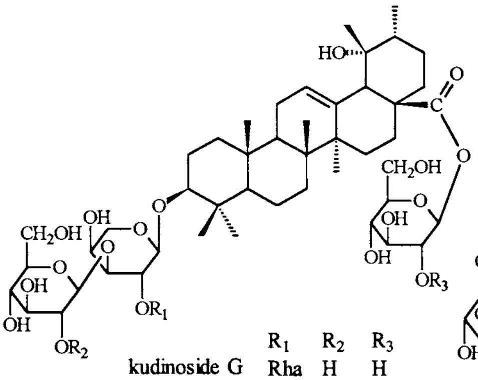
	R
α-kudinlactone	H
kudinoside J	Ara



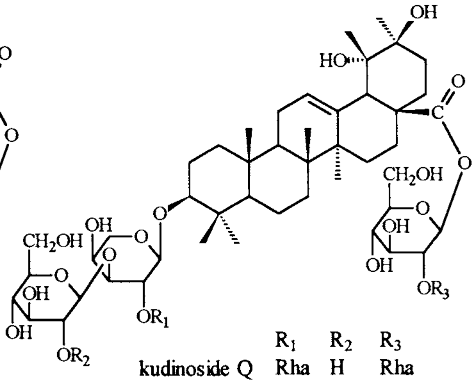
	R1	R2
kudinoside D	Rha	H
kudinoside E	Rha	Glc



	R1	R2	R3
kudinoside N	Rha	H	Rha
kudinoside O	Rha	Glc	H
kudinoside P	Rha	Glc	Rha



	R1	R2	R3
kudinoside G	Rha	H	H
kudinoside K	Rha	Glc	H
kudinoside L	Rha	H	Rha
kudinoside M	Rha	Glc	Rha



	R1	R2	R3
kudinoside Q	Rha	H	Rha
kudinoside S	Rha	Glc	H
kudinoside T	Rha	Glc	Rha

The structures of all saponins are elucidated by chemical and spectral methods. For determine the interglycosyl positions, 2D NMR experiments were used. For example, the

NOESY experiment of kudinoside C showed characteristic cross peaks between the anomeric proton (δ 4.76) of internal α-L-arabinopyranosyl unit and both of the aglycone C-3 proton (δ 3.30) and anomeric proton (δ 6.40) of α-L-rhamnopyranosyl unit, between the anomeric proton of β-D-glucopyranosyl unit (δ 5.13) and both of the α-L-arabinopyraosyl H-3 (δ 4.20) and the anomeric proton (δ 5.24) of terminal β-D-glucopyranosyl unit. These data provided an effective evidence to determine the structure of sugar chain of kudinoside C. The sugar moiety of this saponin to be elucidated as -O-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl(1-3)-[α-L-rhamnopyranosyl (1-2)]-α-L-arabinopyranosyl.

The formation of the triterpenoidal lactonic cycle may be caused by the oxidation of α-amyrin skeleton(Fig.1). It was interesting that each biosynthetic intermediate aglycone has been provided in a glycosidic form from this plant by our isolation. The key intermediate aglycone might have a β-hydroxyl group on C-20 position and belonging to kudinoside Q, S and T. It might be significant that each oxidative step of α-amyrin skeleton can be represented by these aglycones, and possess an important role on the biosynthesis of triterpenoidal skeleton.

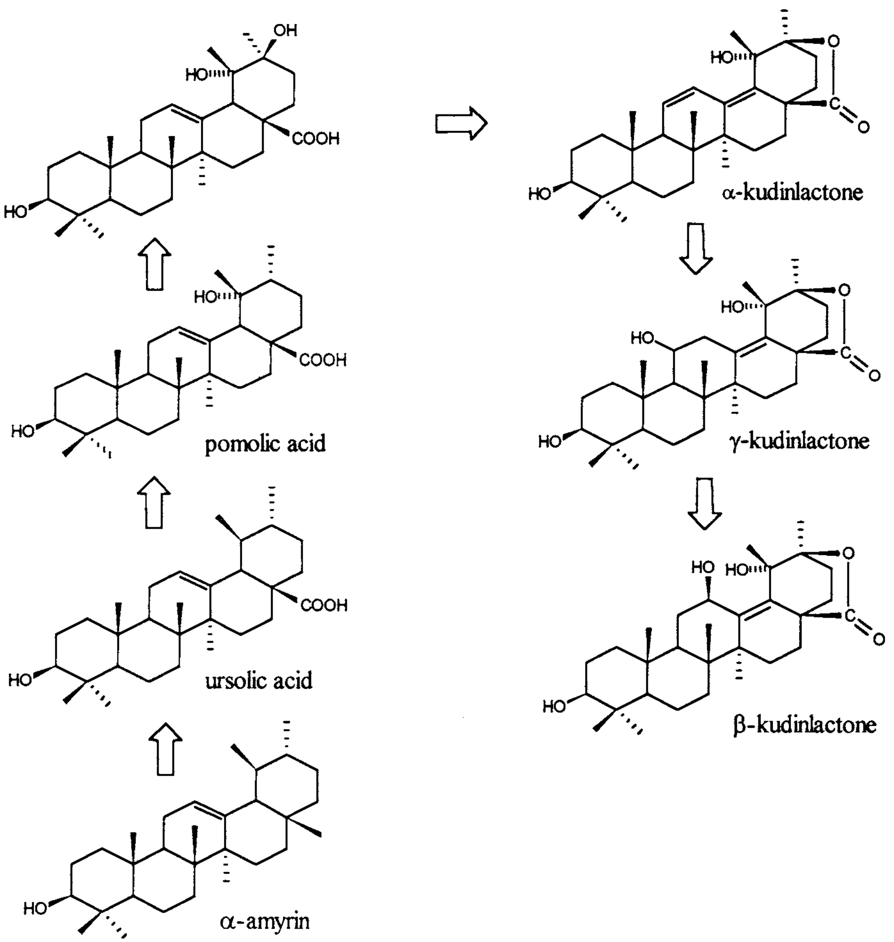
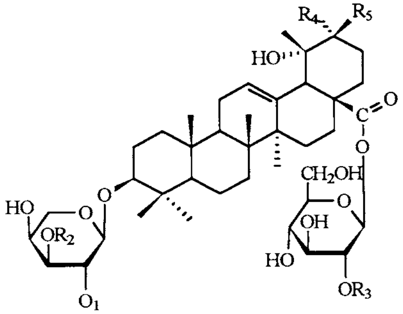
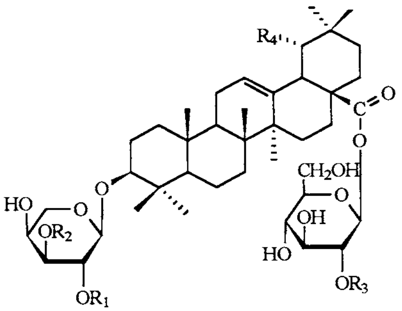


Fig. 1 Biosynthesis pathway of triterpenoid aglycone

Glycosides from *I. latifolia*

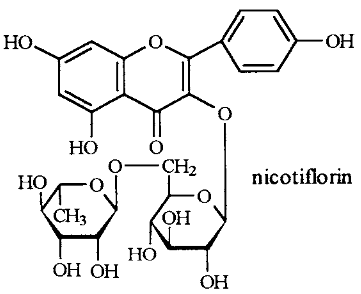
In Hunan and Zhejiang Provinces, the leaves of *I. latifolia* Thunb. are commonly used as the materials of Ku-Ding-Cha. The butanol soluble fraction of the methanol extract of its leaves was repeatedly chromatographed on silica gel column and preparative HPLC to afford sixteen new triterpenoidal saponins, latifolosides A-P, together with two known saponins, kudinosides A and G, and an ionone glycoside, cis-roseoside, as well as a flavonoid glycoside, nicotiflorin[5].



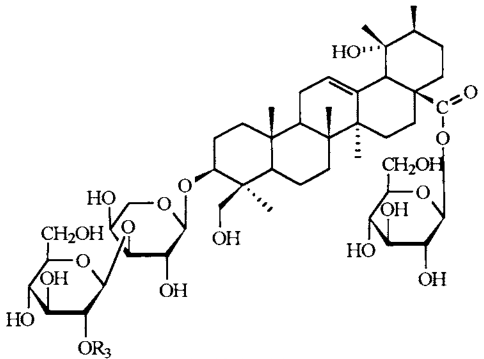


	R_1	R_2	R_3	R_4	R_5
latifoloside A	Rha	H	H	CH_3	H
latifoloside D	Rha	H	H	H	CH_3
latifoloside E	Rha	Glc	H	H	CH_3
latifoloside F	Rha	Glc	Rha	H	CH_3
latifoloside L	H	Glc	H	H	CH_3
latifoloside G	Rha	Glc	Rha	CH_3	H
latifoloside I	H	Glc	H	CH_3	H
latifoloside J	H	Glc	Rha	H	CH_3

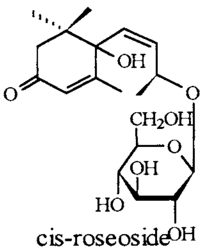
	R_1	R_2	R_3	R_4
latifoloside B	Rha	H	H	OH
latifoloside C	Rha	Glc	H	OH
latifoloside H	Rha	Glc	Rha	OH
latifoloside N	Rha	Glc	H	H
latifoloside O	Rha	H	Rha	H



nicotiflorin



latifoloside K



cis-roseoside

Except kudinoside A, all of these saponins are bisdesmosides and the sugar moieties attached at the both positions of C-3 and C-28 of aglycones, which assignated by glycosylation shift effects of ^{13}C NMR spectra. It is noticed that latifoloside A, D and B; kudinoside G, latifoloside E and C; latifoloside G, F and H are three isomer groups respectively, which aglycones are pomolic acid, ilexgenin B and siaresinolic acid, respectively, and different only on the location of a methyl group in E ring.

The negative ion FAB-MS spectrum of latifoloside F give a quasi molecular ion peak at m/z 1219[M($C_{59}H_{96}O_{26}$)-H]$^-$ and typical fragment ions at m/z 1073[M-146]$^-$, 911[M-146-162]$^-$ and 765[M-2x146-162]$^-$. On acid hydrolysis with 5% H_2SO_4, it afforded glucose, rhamnose and arabinose on the ratio of 2:2:1, and the aglycone which was determined as ilexgenin B. The sugar sequence and interglycosyl linkage positions were achieved by HMBC experiment (Fig.2). The correlative signals between aglycone C-28 and a β-D-glucopyranosyl anomeric proton, between this β-D-glucopyranosyl unit C-2 and an α-L-rhamnopyranosyl anomeric proton indicated that a terminal rhamnosyl unit is linked at the C-2 position of an inner glucosyl unit which is linked at the carboxyl group of C-28 of ilexgenin B. Moreover, correlative signals between aglycone H-3 and an α-L-arabinopyranosyl anomeric carbon, between this arabinosyl C-2 and an α-L-rhamnopyranosyl anomeric proton, between a β-D-glucopyranosyl anomeric proton and the same arabinosyl C-3 indicated that a rhamnosyl and a glucosyl unit located on the terminal position and linked at the inner arabinosyl C-2 and C-3 position, respectively.

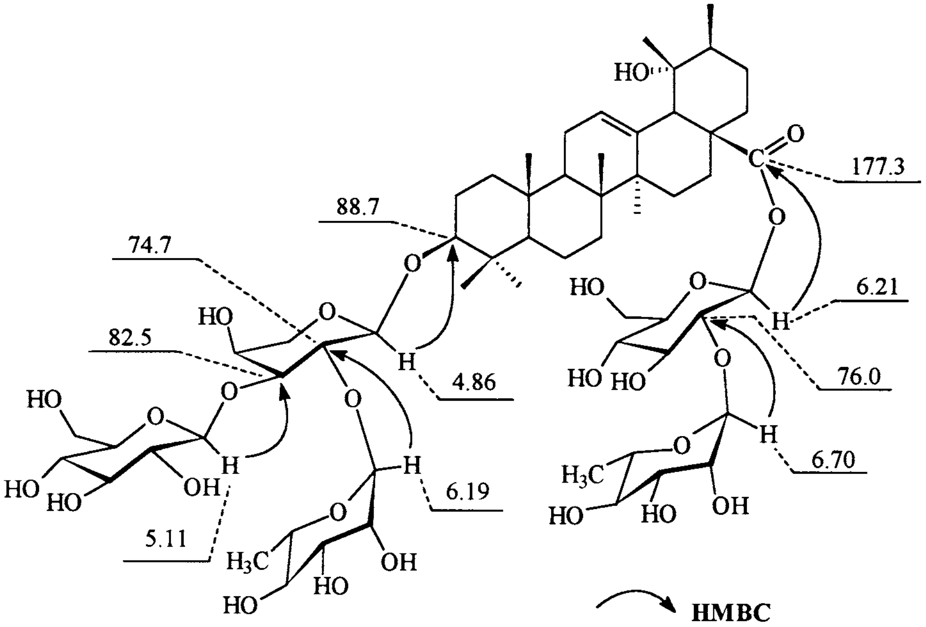


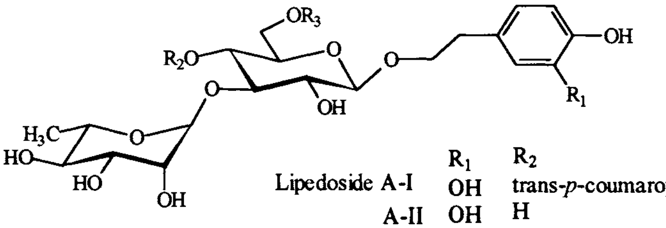
Fig. 2. HMBC of latifoloside F

Glycosides from *L. pedunculare*

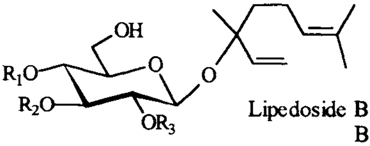
In Sichuan and Hubei province, *L. pedunculare* Rehd., a oleaceous plant, is commonly used as the material of Ku-Ding-Cha. The water soluble fraction of the methanol extract of its dried leaves was fractionated and purified by Diaion HP-20, silica gel and Rp-18 column chromatographies. Two new phenylethanoid glycosides, lipedosides A-I and A-II as well as six new monoterpenoid glycosides, lipedosides B-I to B-VI together with known osmanthuside B, anatolioside and linalool were isolated[2].

Among these glycosides, lipedosides A-I, A-II, B-II, B-III, B-V and B-VI contain a *p*-coumaroyl group linked at the sugar moiety. Usually, the *p*-coumaroyl group is present as a mixture of *cis*- and *trans*-isomers in plants, which led to the difficulty of purifying each isomer. By using a specific separate procedure which keep solvent in low temperature, the pure forms of each isomer were obtained. Lipedosides A-I, A-II, B-III and B-V are *trans*-forms, while, B-II and B-VI are *cis*-forms.

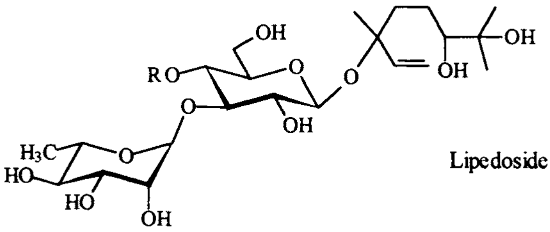
The aglycones of all the monoterpenoid glycosides isolated from this plant are the common constituents of essential oil in plant kingdom. All of sugar moiety of these glycosides linked at an asymmetric carbon atom of aglycone's C-3 hydroxyl group. Through a enzymatic hydrolysis, lipedoside B-I gave (R) and (S) mixture of linalool (R:S=13:87, detected by GC). Moreover, both of B-II and B-III were transformed to B-I after alkaline hydrolysis(Fig.3). Therefore, these monoterpenoid glycosides could be characterized as a mixture of 3(R) and 3(S) forms.



	R_1	R_2	R_3
Lipedoside A-I	OH	trans-*p*-coumaroyl	H
A-II	OH	H	trans-*p*-coumaroyl
Osmanthuside B	H	trans-*p*-coumaroyl	H



	R_1	R_2	R_3
Lipedoside B-I	H	Rha	H
B-II	cis-*p*-coumaroyl	Rha	H
B-III	trans-*p*-coumaroyl	Rha	H
Anatolioside	H	H	Rha



	R
Lipedoside B-IV	H
B-V	cis-*p*-coumaroyl
B-VI	trans-*p*-coumaroyl

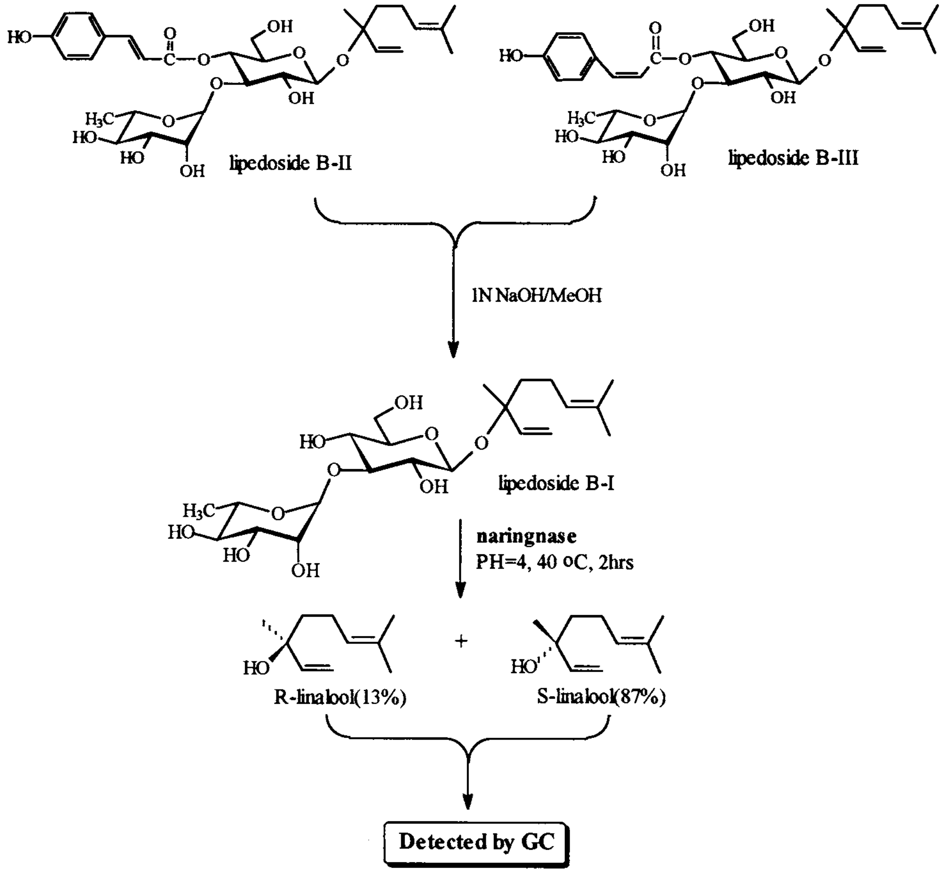
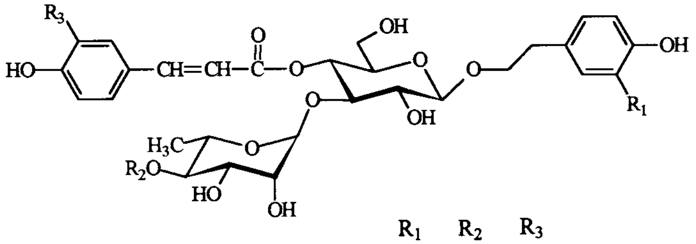


Fig. 3 Hydrolysis of isomers and determining (R,S) linalool

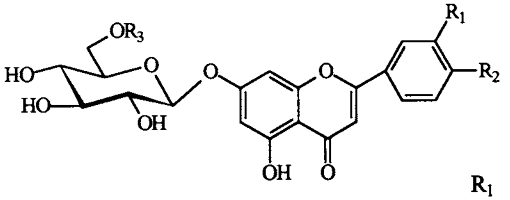
Glycosides from *L. purpurescens*

The leaves of *L. purpurescens* Y.C.Yang as the materials of Ku-Ding-Cha are only used in the northeast of Yunnan province. Two new phenylpropanoid glycosides, ligupurpuroside A and B together with the Z/E isomer mixture of 2-(3,4-hydroxyphenyl) ethyl (3-O-α-L-rhamnopyranosyl)(4-O-coumaroyl)-O-β-D-glucopyranoside(glycoside A), the mixture of osmanthuside B and its *cis* isomer (glycoside B), and acteoside were isolated from the methanol extract of its dried leaves. Three known flavonoid glycosides, luteolin-7-O-glucoside, cosmosiin and rhoifolin were also obtained from this plant[1].

It is noticed that the content of acteoside in this plant is very high by HPLC analysis and all of the flavonoid glycosides are isolated from the genus *Ligustrum* for the first time.



	R_1	R_2	R_3
Acteoside (E)	OH	H	OH
Ligupurpuroside A (E)	OH	Rha	OH
Ligupurpuroside B (E)	H	Rha	H
glycoside A (Z/E)	OH	H	H
glycoside B (Z/E)	H	H	H



	R_1	R_2	R_3
Luteolin-7-glucoside	OH	OH	H
Cosmosiin	H	OH	H
Rhoifolin	H	OH	Rha

Biological Activties

The diversity of the original materials of Ku-Ding-Cha and the structures of their glycosides provided an opportunity for search new bioactive lead compounds from natural resources. It is well known that free radical induced lipid peroxidation damage as a critical initiating event leading to cell injures[6]. The lipid peroxidative reaction may disturb the fine structure of the cells and may thus effect the permeability and function of the membranes. These processes may cause irreversible damage of the cells and may initiate and/or promote the pathogenesis of certain disease[7-8]. Therefore, it has important significance to find effective antioxidants and free radical scavengers for preventing and treating diseases.

According to a primary screening test, the anti-PAF activity of some triterpenoid saponins from *Ilex* and the antioxidant activity of phenylethanoid glycosides from *Ligustrum* had been found. For further evaluate against lipid peroxidative activity of acteoside, ligupurpuroside A and B, we successfully induced the lipid peroxidation of the rat liver microsomes with Fe^{2+}-cysteine and the generating system of oxygen free radicals, as well as LDL oxidation by the electron supporter Cu^{2+}.

The effect of phenylethanoid glycosides on the rat liver microsomal lipid peroxidation

The results indicated that acteoside, ligupurpuroside A and B dose-dependently inhibit MDA formation induced by Fe^{2+}-cysteine. The inhibitory rate(%) of acteoside and ligupurpuroside B is close to Vitamin E, and better than ligupurpuroside A.(Table 2)

Table 2. The inhibitory effect of three phenylethanoid glycosides on rat liver microsome lipid peroxidation

	Inhibitory Rate(%)			
ug/ml	acteoside	ligupurpuroside B	ligupurpuroside A	Vit E
52	92.1	90.4	---	---
26	74.2	72.4	67.7	75.4
13	60.3	62.1	52.5	---
7	50.1	49.4	40.0	---

The inhibitory effect of glycosides on LDL oxidation

These three glycosides can increase the lag time before the onset of LDL oxidation remarkably as compared with the control. The effect is concentration-dependant, and better than the same concentration of Vitamin C, a positive control. Among them, acteoside and ligupurpuroside B showed a better antioxidant activity (Table 3).

Table 3. The effect of three phenylethanoid glycosides on the lag time of LDL oxidation modification

	Lag Time (min)		
	acteoside	ligupurpurosideA	ligupurpuroside B
control	84.9	56.6	74.6
Vit C	105.4[a]	66.9[a]	131.1[b]
400 ng/ml	complete	126.0	153.0
200	153.0	90.0	144.0
100	136.3	73.0	101.0
50	128.6	64.0	86.0

[a] Final concentrtion = 140ng/ml [b] Final concentrtion = 280ng/ml

Though, there have been many researches about the antioxidative effect of polyphenols[9,10], phenylethanoid glycosides as a kind of stronger antioxidative agent had not been much reported. The antioxidative properties of acteoside, ligupurpuroside A and B may be related to their multiple phenolic hydroxyl groups. The action of acteoside and ligupurpuroside B are more potent than that of ligupurpuroside A. That might not only due to the amount of the phenolic hydroxyl groups, but also for the structure of sugar moiety. The function of antioxidative action might be through the interruption of the free radical chain reaction by accepting electrons.

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