한국농약과학회지 제6권 제1호(2002)
The Korean Journal of Pesticide Science
Vol. 6, No. 1, pp.9~15(2002)

〈Review〉

The Insecticide Constituents of Several Celastraceae Plants

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Abstract : The insecticide constituents of several Celastraceae family plants including *Celastrus angulatus, Celastrus orbiculatus, Celastrus flagellaris, Euonymus verrucosides, Euonymus forunei* and *Euonymus phellomana* were studied by bioassay-guided fractionation. All structures of sesquiterpene polyol esters and alkaloids have been elucidated by UV, IR, MS, NMR and x-ray diffraction evidences. These compounds exhibited insecticidal, antifeedant and narcotic activities against *Mythimna separata.*(Received February 12, 2002; accepted March 22, 2002)

Keywords : antifeedant, Celastraceae, *Celastrus angulatus, Celastrus orbiculatus, Celastrus flagellaris, Euonymus verrucosides, Euonymus forunei, Euonymus phellomana;* sesquiterpene polyol ester and alkaloid, insecticidal, narcotic activities.

Introduction

Plants of the family Celastraceae produce various β -dihydroagarofuran sesquiterpene polyol esters and alkaloids, of which some exhibit insecticidal, insect antifeedant, antitumor, Anti-HIV activity and reversing multidrug resistance in cancer cell (Bruning and Wagner, 1978; Takaishi *et al,* 1993; Liu *et al,* 1990; Tu *et al,* 1990; Takaishi *et al,* 1993; Kim *et al,* 1999; Duan *et al,* 2000). The IC_{50} of celaglaumin against L1210 and P-388 cell were 2.22 and 4.12 μg/mL, respectively. Celanguilin I and angulatin A exhibited strong insecticidal and antifeedant action against *Mythimn separata* at the concentration of 25-50 μg/mL. Triptonine B had potent anti-HIV activity with an EC_{50} value of 0.1 μg/mL. Orbiculin E and F, triptogelin C-1 and Ejap-2 were shown to be more active than verapamil in reversing vinblastine resistance in multidrug-resistant KB-V1 cells. Some Celastraceae plants such as *Celastrus angulatus, Celastrus orbiculatus* and *Tripterygium wilfordii* are traditional insecticidal plants, which are widely distributed and used in Peoples Republic of China. Extracts of theses plants exhibited insecticidal, nacotic and insect antifeedant activity against several insect species[8]. In previous study, several antifeedant and insecticidal compounds celangulins I-V were isolated from the root bark of *Celastrus angulatus* (Wakabayashi *et al,* 1988; Wu *et al,* 1992). The other researchers also elucidated some new sesquiterpene polyol esters and alkaloids isolated from the root bark and leaves of *Celastrus angulatus* (Liu *et al,* 1991; Liu *et al,* 1995; Wang *et al,* 1991). This paper deals with the isolation and structure elucidation along with the insecticidal, antifeedant, narcotic activities of the isolated compounds.

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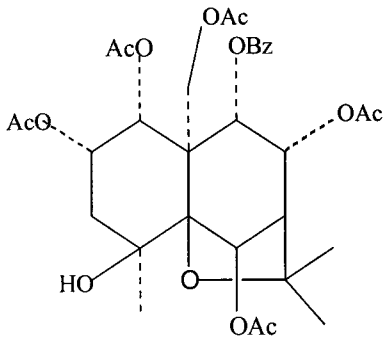
Sesquiterpene polyol esters and alkaloids from the family Celastraceae plants in China

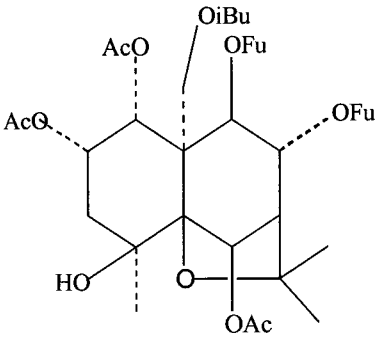
1. Sesquiterpene polyol esters from the root bark and leave of *Celastrus angulatus*

The powdered root bark of Chinese bittersweet, *Celastrus angulatus* Maxim., has been used traditionally to protect plants from insect damage. Investigations of the powdered root bark have demonstrated activity against several insect species, such as cucumber beetle, *Aulacophora femoralis,* cruciferous leaf beetle, *Colaphellus bouringi,* willow leaf beetle, *Plagiodera versicolora,* cabbage sawfly, *Aathalia flacca,* Hawaiian beet web-worm, *Hymenia recurvalis,* imported cabbagege worm, *Pieris rapae,* a tent caterpillar, *Malacosoma neustria testacea,* migratory locusts, *Locusta migratoria migratorioides* and *Locusta migratoria manilensis.*

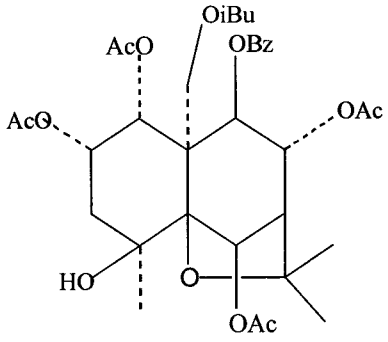
Except celangulin I-V, several other components was isolated subsequently by other researchers, no significant biological activity for these compounds was reported. In continuation of a search for biological active compounds and QSAR research, continued activityguided fractionation has led to isolation of eight new b-dihydroagrofuran sesquiterpene polyol esters



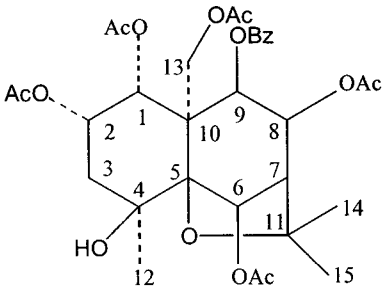
Celangulin I



Celangulin II



Celangulin III



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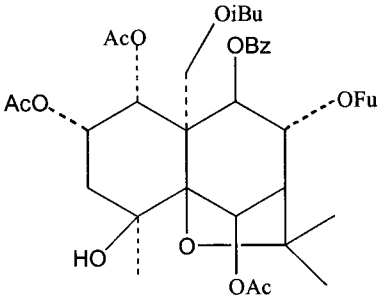

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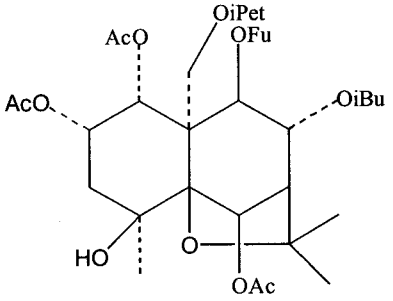

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(**1-8**) from the root bark, and two new (**9**, **10**) along with two known β-dihydroagrofuran sesquiterpene polyol esters (celangulin IV and V) from the leave of *Celastrus angulatus*. The structures of ten new sesquiterpene esters is:

2. Sesquiterpene polyol esters from *Celastrus orbiculatus*

Celastrus orbiculatus Thunb is *Celastrus* genus plant, distributed widely in Peoples Republic of China. Its root, stem, fruit and leaf were used as traditional medicine and insecticide in China. C. R. Smith investigated the chemical constituents of seed oil of *C. orbiculatus* in 1976, identified the structures of two sesquiterpene polyols Celorbicol and Isocelorbicol by UV, IR, MS, NMR and x-ray diffraction. The structures of sesquiterpene polyol esters were not determined correctly only by comparing their NMR data with Celorbicol and Isocelorbicol. J. W. Huffman developed two stereoselective synthesis routines for Isocelorbicol. However, there was no report about the biological activity of its constituents.

We carefully investigated the sesquiterpene polyol esters of seed oil of *C. orbiculatus*, isolated and elucidated two new (**11**, **12**) and seven known β -dihydroagrofuran sesquiterpene polyol esters. The structures **13** and **14** were revised as **15** and **16** by the results of x-ray diffraction.

3. Sesquiterpene polyol esters from *Celastrus flagellaris*

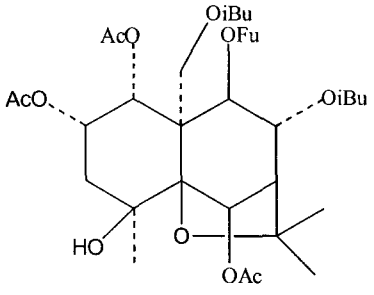
Celastrus flagellaris Rupr. is *Celastrus* genus plant, distributed northeastern district and Zhejiang province in Pepoles Republic of China, and Korea. Its root, stem, fruit and leaf were used as traditional medicine for rheumarthritis in China. Until now there was no report about the chemical constituents and biological activity of its constituents. Due to the insecticide activity of β-dihydroagrofuran sesquiterpene polyol esters and alkaloids isolated from *C.angulatus*, *C. orbiculatus* and other Celastraceae family plants.

We carefully investigated the sesquiterpene polyol esters of seed oil of *C. flagellris*, isolated and elucidated two new (**17**, **18**) and eight known β-dihydroagrofuran sesquiterpene polyol esters.

Compound **17** is the epimer of Ejap-3 (**19**) at C-9, while compound **18** is the ester of Isolorbicol and the first report of two cinnamate esters in the same molecule of a β-dihydroagrofuran sesquiterpene.

4. Sesquiterpene alkaloids from *Euonymus verrucosides*

Euonymus verrucosides Loes is Euonymus genus plant, distributed widely in China. Until now there was no report about the chemical constituents and biological activity of its constituents. Due to the insecticide activity of β-dihydroagrofuran sesquiterpene alkaloids isolated from the family Celastraceae plants, such as evonine, wilfordine, wilforine, wilforging,



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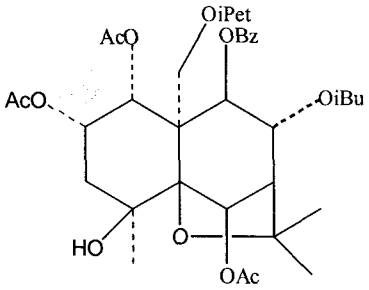

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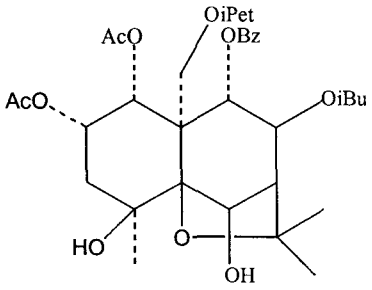

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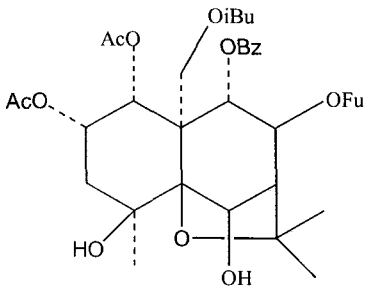

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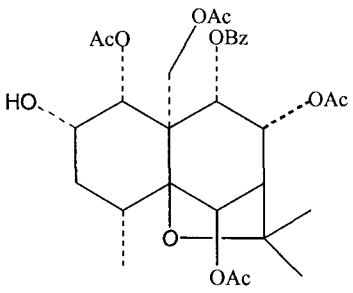

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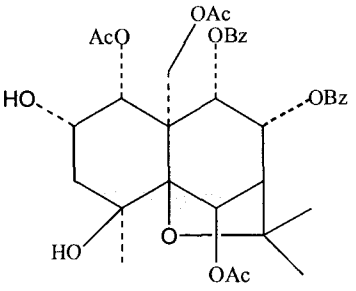

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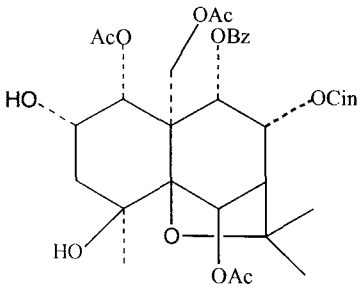

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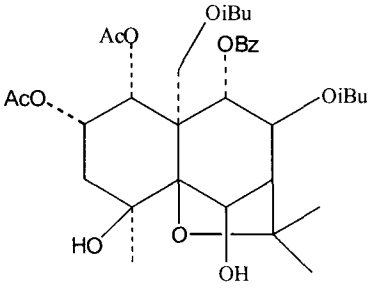
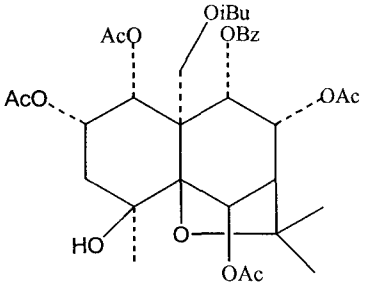
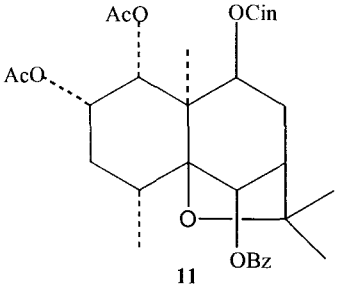


celangulin V



celangulin IV



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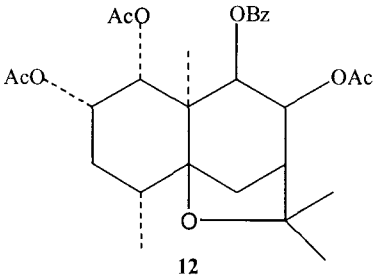

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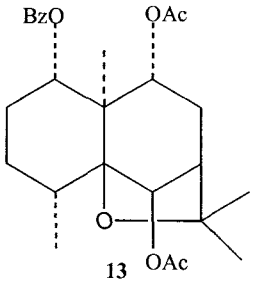

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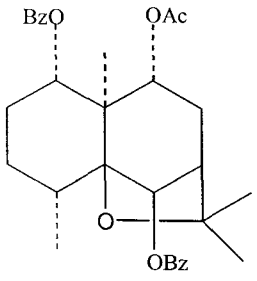

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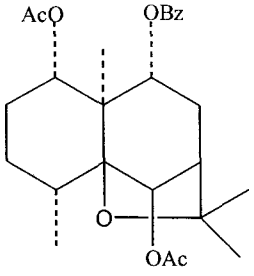

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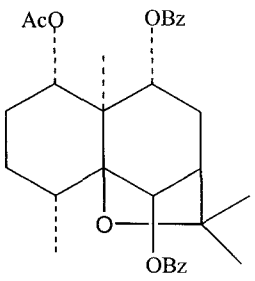

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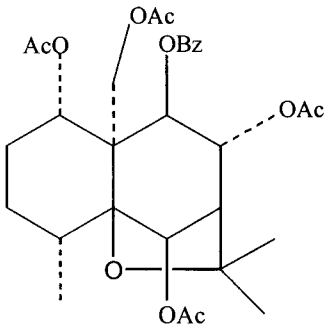

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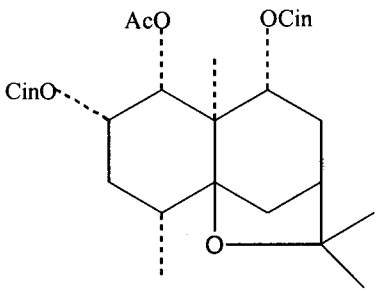

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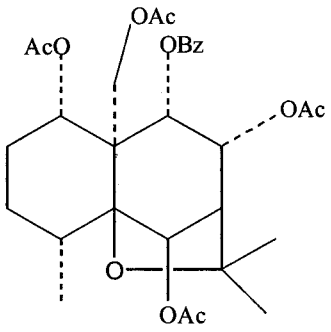

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wilfornine and forrestine, we carefully investigated the sesquiterpene alkaloids of the root bark of *E. verrucosides* by activity-guided fractionation, isolated and elucidated two new (**20, 21**) and one known (**22**) b-dihydroagrofuran alkaloids.

5. Sesquiterpene alkaloids from *Euonymus forunei*

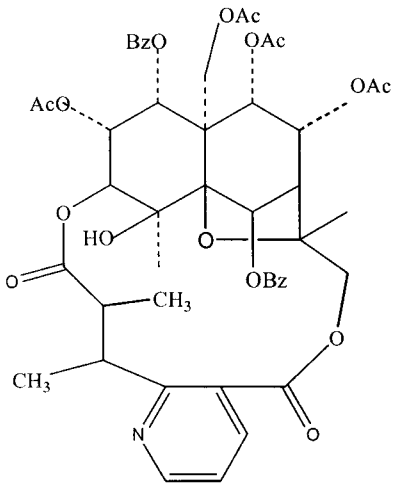
Euonymus forunei (Tuec) Hard-Mazz. is Euonymus genus plant, distributed widely in China. Until now, dulcitol, tritriacontane, friedelaone, epifriedelanol, friedelanol, β-sitosterol and only one β -dihydroagrofuran sesquiterpene polyol ester Ejap-5 have been isolated from leaves and seed of *E. forunei*. Extract of root bark of this plant showed insecticide activity against several insects. We carefully investigated the sesquiterpene alkaloids of the root bark of *E. forunei* by activity-guided fractionation, isolated and elucidated one new b-dihydroagrofuran alkaloids which has the same structure as **21**. Compound **21** has different substitute groups at C-1 and C-2 from wilforine (**23**) and is the epimer of Euojaponine F (**24**) at C-8.

6. Sesquiterpene alkaloids from *Euonymus phellomana*

Euonymus phellomana Loes is Euonymus genus plant, distributed in Henan, Sichuan, Shanxi, Gansu and Ningxia province of China. Its stem and leaf were traditional medicine in China.

Until now only two β-dihydroagrofuran sesquiterpene polyol esters were isolated and identified from its seed oil. There was no report about the chemical constituents and biological activity of its constituents of root bark of this plant. We carefully investigated the sesquiterpene alkaloids of the root bark of *E. phellomana* by activity-guided fractionation, isolated and elucidated one new (**25**) and one known β-dihydroagrofuran alkaloids, which has the same structure as **22**.

Based on the above results, we have isolated seventeen new and twenty known β-dihydroagrofuran sesquiterpene polyol esters and alkaloids from celastraceae plants: *Celastrus angulatus, Celastrus orbiculatus, Celastrus flagellaris, Euonymus verrucosides, Euonymus forunei* and *Euonymus phellomana.*



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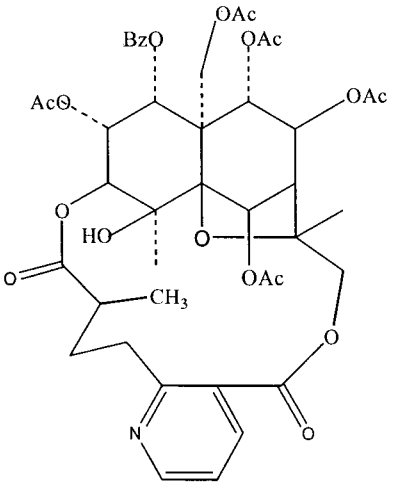

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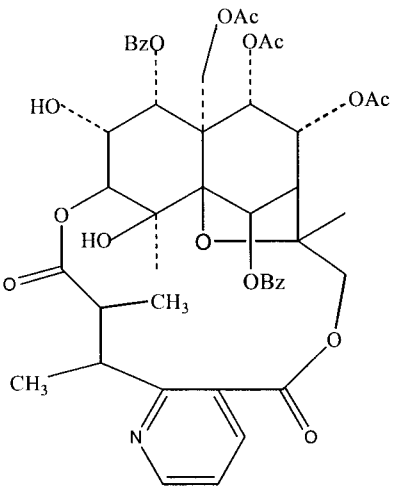
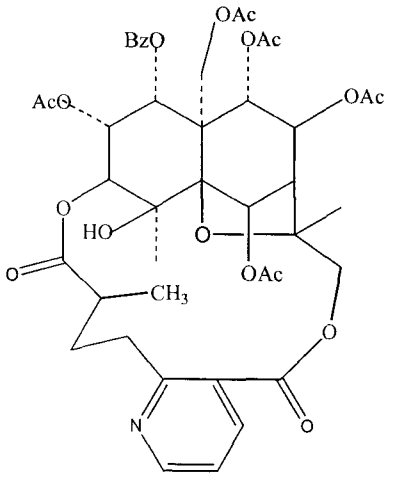


22 (Euojaponine C)



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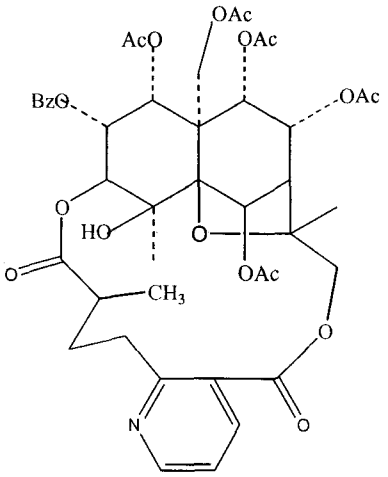
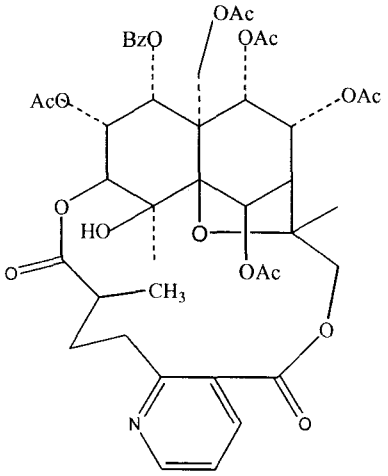


23 wilforine



24 Euojaponine F

The insecticidal, insect antifeedant, narcotic activities of the isolated novel compounds

1. Experimental methods for bioassay

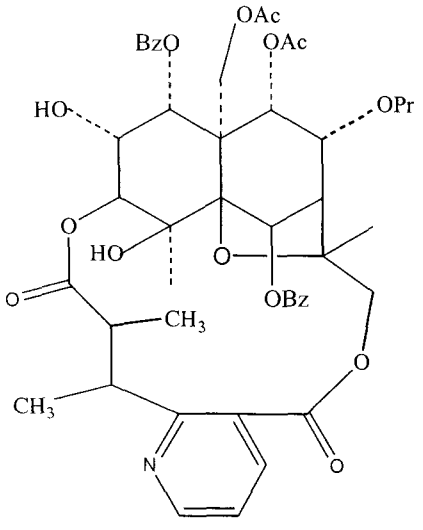
Leaf of known area was treated with known amounts of test samples dissolved in acetone. The 5th instar larvae of *Mythimna separata* were fed with the discs for 12-24 h. The areas eaten were measured under a binocular microscope by counting 1-mm squares exposed when the partially eaten disc was placed on a circle the exact size of the disc drawn on mm-ruled paper. After 24h, the numbers of knocked-down larvae (Symptoms: the larvae were narcotized and could not move; the bodies were immobilized and very soft; and the response disappeared completely) or dead larvae were recorded, and the toxicity was ascertained by estimating the median knock-down dose or median lethal dose of test sample. The antifeedant rate was calculated by the two following equation:

antifeedant rate(%)=(controlled eaten areas − treated eaten areas)/controlled eaten areas×100% ..... (1)

antifeedant rate(%)=(controlled added weight − treated added weight)/controlled added weight×100% ..... (2)

2. The insecticidal or nacotic activities of novel compoun

It is very interesting to note that the insects treated with compounds **1**, **2**, **21**, **22** and **25** were immobilized without convulsive symptoms, while the insects treated with compounds **3-8** lost fluid with slight convulsive symptoms. These results showed that compounds **1**, **2**, **21**, **22** and **25** exhibited nacotic action to *Mythimna separata* while compounds **3-8** exhibited insecticidal action to *Mythimna separata*. We found compounds with different substitute ester groups or stereochemistry at C-8 and C-9 showed different biological activities. Detail structure-activity relationship is under way (Table 1).



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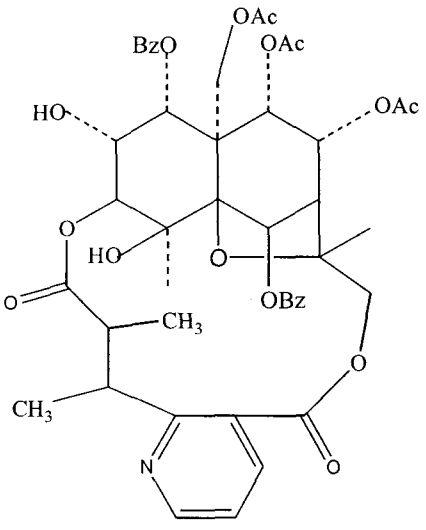


22 (Euojaponine C)

Table 1. The insecticidal or nacotic activities against the larval of *Mythimna separata* (ig/g)

No.	1	2	3	4	5	6	7	8	10	21	22	25
LD_{50} or ND_{50}	159.8	58.9	91.4	271.5	168.8	135.3	73.3	388.0	215.8	21.6	102.5	168.5

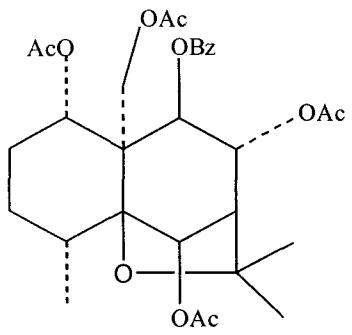
Table 2. The insect antifeedant activity of several compounds

No.	Conc. (mg/L)	Treated eaten area (mm^2)	Controlled eaten area (mm^2)	Antifeedant rate (%)
19	250	3	28.2	89.4
17	250	41.2	111.3	63.0
26	250	35.2	76	53.7
15	250	35.0	288	87.8
27	250	100	344	70.9
28	250	49	256	80.9
19	500	4	57.5	93
17	500	23.3	109	78.6
26	500	3	91.3	96.7
15	500	30	299	90.0
27	500	44	247	82.2
28	500	13	371	96.5

3. The insect antifeedant activity of novel compounds and similar analogues

The insect antifeedant activity of compounds **15, 17, 19, 26, 27, 28** has been tested at the 500 and 250 $\mu g/mL$ concentrations with acetone solution, the controlled sample is pure acetone. Results at the table 2 showed this kind of compounds exhibited insect antifeedant activities to *Mythimna separata* at given concentration. Sometime the symptoms is the same as insecticidal or nacotic action.

The preliminary structure-activity relationship showed that: 1. The existing ester groups or not at C-13 did not influence greatly the activity; 2. Benzoate or cinnamate at C-9 contributed to the antifeedant activity without characteristic difference; 3. The existing ester groups or not at C-6 had some influence



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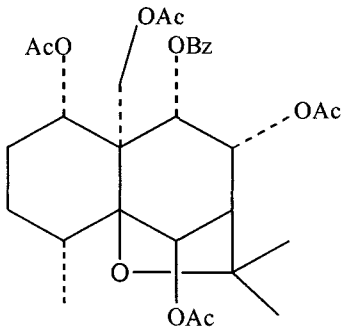

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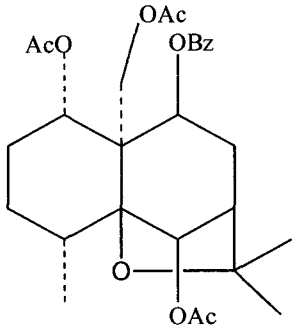

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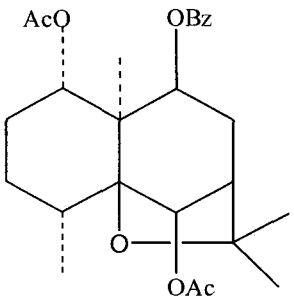
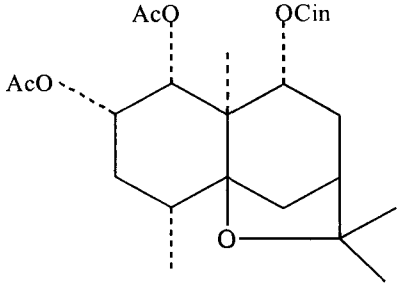
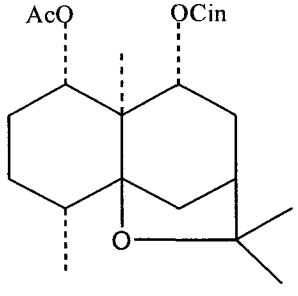


15



27



28

at low concentration; 4. Compound **19** is an epimer of **17**, only the stereochemistry at C-9 different from Compound **17**. The antifeedant rates of **19** were higher than the rates of **17** at two concentrations. These results may relate with their different orientation of ester groups at C-9.

Conclusion

Eighteen new insecticidal and antifeedant constituents have been isolated from the Celastraceae family plants including *Celastrus angulatus, Celastrus orbiculatus, Celastrus flagellaris, Euonymus verrucosides, Euonymus forunei* and *Euonymus phellomana* by bioassay-guided fractionation. All structures of sesquiterpene polyol esters and alkaloids have been elucidated by UV, IR, MS, NMR and x-ray diffraction evidences. These compounds exhibited insecticidal, antifeedant and narcotic activities against *Mythimna separata.*

Acknowledgement

These researches were founded financially by National Natural Science Foundation of China and Doctorial Research Foundation of Education Administration of China. Thanks also are given to Prof. Chen Fuheng, Zhou Changhai, Li Shouqiao and Dr. Shen Jizhong of China Agricultural University, and Prof. Liu Huixia, Yang Jingqiang, Dr. Zhu Jinbo, Dr. Zhou Wenming, Dr. Hu Zhaonong and Dr. Ji Zhiqing of Northwestern Agricultural University. The authors thank the Microchemistry Institute of Beijing, College of Chemistry of Peking University and the Chemistry Institute of Chinese academy of Science for measuring the HRFABMS, EIMS, NMR and x-ray diffraction data. Prof. Norman G. Lewis, Prof. Richard G. Powell and Prof. A. Douglas Kinghorn, the editors of the journal Phytochemistry and Journal of Natural Products, are gratefully acknowledged for their kind helps.

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