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**Note**

# Antifeedant Activities of *Ginkgo biloba* L. Components against the Larva of *Pieris rapae crucivora*

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The ginkgo tree, *Ginkgo biloba* L. that is called a "living fossil", is one of the oldest of living plants and has remained unchanged throughout 200 million years. To explain the longevity of this tree, its resistance to various pests such as insects, bacteria, viruses and fungi has been previously reported.[1] However, chemical ecological studies on major *Ginkgo biloba* components have been neglected. In the search for pest insect control agents based on natural products, we have examined *Ginkgo biloba* L. (Ginkgoaceae) which is relatively free from insect attack.

We found that a crude methanol extract of *Ginkgo biloba* leaves possesses antifeedant activity against the third-instar larvae of cabbage butterfly (*Pieris rapae crucivora*) by using a leaf disk bioassay.[2] The glandless leaves of cabbage (*Brassica oleracea* L. var. *capitata* L.), a favored host of the cabbage butterfly, were used as leaf disks. Leaf disks (1 $cm^2$) were punched out, randomized, and arranged (12 disks/dish) in a circle on moistened filter paper in polyethylene form grids inside glass petri dishes ($100 \times 15$ mm). Alternating disks were treated on their upper surface with either 50 $\mu$l of acetone or with test compounds dissolved in 50 $\mu$l acetone. Two third instar larvae were then placed in the disks at 25°C in a dark. After 10～16 hr, the larvae were removed, and the weight of sample disk was compared to that of the control disk. Each treatment was repeated 2 times and the antifeedant activity was determined as the mean of feeding inhibitory ratio: $100 - 100 \times$ (consumed amount of sample disk)/(consumed amount of control disk).[3]

Further separation was carried out monitoring with this bioassay. The crude methanol extract was dispersed into water and the aqueous suspension was extracted with chloroform and then with methyl ethyl ketone. A chloroform extract showed 80% inhibition at 500 $\mu$g/disk and was fractionated by silica gel column chromatography and/or reversed phase ($C_{18}$) liquid chromatography to give the following known compounds as active components: the phenolic acids,[4] 6-pentadecenylsalicylic acid (**1**) and 6-heptadecenylsalicylic acid (**2**), and the bitter sesquiterpene, bilobalide (**3**).[5] The methyl ethyl ketone extract, showed more potent activity (98% inhibition at 500 $\mu$g/disk) than chloroform extract, was purified by the combined technique of column chromatography with counter current chromatography. Known bitter terpenoids, ginkgolide A (**4**), B (**5**)[6] and bilobalide, were major active principles. All these known chemicals were identified by spectroscopic methods (EI-MS, $^1H$ and $^{13}C$ NMR) and chromatographic methods. Two new flavone glycosides with bitter taste were also isolated from the methyl ethyl ketone extract as weak antifeedants, and these structures were established as kaempferol 3-*O*-$\alpha$-(6‴-*p*-coumaroylglucosyl-$\beta$-1,2-rhamnoside) (**6**) and

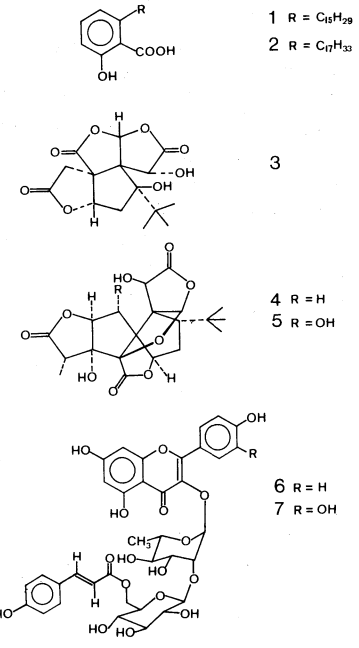


TABLE I. ANTIFEEDANT ACTIVITIES OF *Ginkgo biloba* COMPONENTS AT 500 $\mu$g/disk

| Test compound | Feeding inhibitory ratio (%) |
|---|---|
| 6-Pentadecenylsalicylic acid (**1**) | 63 |
| 6-Heptadecenylsalicylic acid (**2**) | 69 |
| Bilobalide (**3**) | 90 |
| Ginkgolide A (**4**) | 98 |
| | 57[a] |
| Ginkgolide B (**5**) | 81 |
| Kaempferol glycoside (**6**) | 54 |
| Quercetin glycoside (**7**) | 42 |
| Biflavones (**8**～**11**) | <11 |

[a] Datum at 50 $\mu$g/disk.

quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,2-rhamnoside) (**7**).[7] Although biflavones, one of the major class of components in *Ginkgo biloba*, were isolated from the active chloroform extract and identified as bilobetin (**8**), isoginkgetin (**9**), ginkgetin (**10**) and sciadopitysin (**11**),[8] they were not active principles.

Table I indicates the antifeedant activities of purified chemicals at 500 μg/disk. Apparently, leaf disks treated with ginkgolides or bilobalide were almost protected from feeding. The most potent was ginkgolide A, which showed more than 50% of feeding inhibitory activity even at 50 μg/disk. To our knowledge, none of biological activities of ginkgolides or bilobalide against insects has been reported so far. Phenolic acids showed moderate activities. Since anacardic acid, similar phenolic acid, is known as a insect oviposition inhibitor,[9] the observed feeding deterrent effect may concern with oviposition behavior. Although the biflavones did not show any activity, flavone glycosides inhibited the feeding of the larvae. The content of biflavones, in the leaves are more than three times higher in autumn than in spring and summer.[10] In contrast the content of other three classes of chemicals, phenolic acids, terpenoids and flavone glycoside, decrease in autumn.[11] The feeding deterrent action of these three different types of secondary metabolites may represent a behavioral defense barrier in *Ginkgo biloba*.

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