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Identification of natural insecticidal compound in medicinal plants against diamondback moth

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Abstract : Insecticidal potentials of polar and non-polar fractions obtained from 84 medicinal plants were screened against five major agricultural insects. Based on the primary and secondary screening results, non-polar fraction of *Atractylodes koreana* Kitam. rhizomes was selected to isolate and identify an active compound effective to diamondback moth (*Plutella xylostella* L.) larvae. Counter-current distribution separation on the non-polar fraction and TLC and spectroscopic analyses (GC-MS and ^{1}H- and ^{13}C-NMR) revealed that molecular formula of the active compound was $C_{15}H_{22}O$ known as a sesquiterpenoid 4,11-selinadien-3-one (α-cyperone). However, α-cyperone was not detected in the non-polar fractions that showed high insecticidal potential against the diamondback moth. Although α-cyperone has been first identified from *Cyperus rotundus*, the compound did not occur in *C. rotundus* cultivated in Korea.(Received December 18, 1998, accepted April 30, 1999)

Key words : *Atractylodes koreana*, α-cyperone, diamondback moth.

Introduction

Numerous natural compounds of plants with pesticidal potential have been screened and identified. Some of them have been successfully exploited as pesticides and commercially used. The most successful use of a plant-derived product as a pesticide is probably that of the pyrethroids. They have been developed from the six terpenoid esters (pyrethrins) which are responsible for the insecticidal activity of several *Chrysanthemum* species (Green *et al.*, 1987).

Many sesquiterpenoids show various biological activities. Some plant-derived sesquiterpenoids have been found to possess insecticidal activity. Caryophyllene is an aphid repellent (Gregory *et al.*, 1986) and also has antifeedant activity against cabbage butterfly larvae (Yano, 1987). The epoxide of this compound inhibits *Heliothis virescens* larval growth (Stipanovic *et al.*, 1986). α-Santonin is structurally much resemble to α-cyperone and is well-known anthelmintic component obtained from various *Artemisia* species (Dewick, 1997). All of the plant-derived compounds that have been characterized as having pesticidal activity are secondary products or metabolites of plants. In this experiment, we screened insecticidal activities of extracts of various medicinal plants. Out of them, rhizome extract of *A. koreana* (Korean name Chamsapju) belonging to Asteraceae provided very high mortality against the diamondback moth larvae. Therefore, we tried to identify the active compound from the extract and examined presence of the compound in other medicinal plant extracts whose insecticidal activity against the larvae was being investigated.

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Materials and methods

Preparation of plant extract

Plant materials employed in this study were randomly purchased from the oriental medicinal herb markets. Dry materials (ca. 1kg) were ground in a mill and refluxed with 2L of 70%-methanol for 2 h in a heating mantle. The crude extract was concentrated *in vacuo* and separated by counter-current distribution into *n*-hexane and water (aqueous) fractions using separatory funnels. Non-polar components from the aqueous fraction were further removed with dichloromethane and toluene in order. Both the non-polar (*n*-hexane) and the polar (water) fractions were then concentrated *in vacuo* and determined their yields. They were used to determine an insecticidal potential against the diamondback moth larvae.

Screening for the diamondback moth larvae

Fresh cabbage leaf disks (5-cm diameter) were dipped in appropriate concentrations of the extract for 30 seconds and dried in a hood. Primary screening test was conducted at the application rate of 5000 ppm, which was diluted from the final concentrated fraction of the respective plant extracts. The disks were placed in a 5 cm-plastic petri dish and ten third-instar larvae of diamondback moth were transferred into the dish. After sealing with the cover to avoid release of the larvae, the dishes were kept at 25℃ with 60% relative humidity under continuous light condition. Larval mortalities were recorded at 24 and 48 h after treatment. All tests were replicated three times. Secondary screening test was carried out at the gradually decreased application rates of the fractions selected after the primary screening test in the same manner described above.

Identification and determination of active compound

Non-polar fraction of *A. koreana* that showed the highest mortality at the lowest application rate among the tested fractions was selected for identification of the active compound. The active fractions were separated in silica gel column chromatography (Kiesel gel 60, 70~230 mesh, Merck) with successive elution of *n*-hexane, ethyl ether, ethyl acetate, and methanol (Fig. 1). Each fraction was individually collected, concentrated, and bioassayed with the diamondback moth larvae. The active fraction was further separated by preparative thin layer chromatography (TLC, Kiesel gel 60 F_{254} 20×20 cm, 0.5 mm thickness, Merck) using *n*-hexane:acetone (9:1, v/v) solvent system. After developing the TLC plate, each region indicating one or more compounds according to the R_f values was scrapped, eluted with acetone, concentrated and bioassayed.

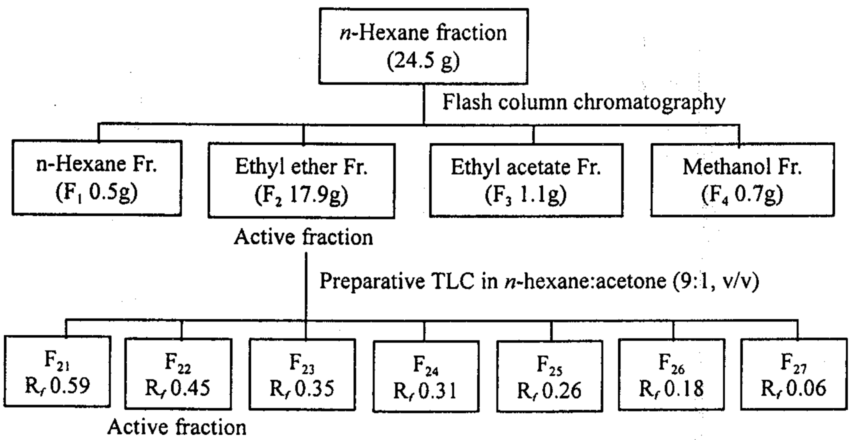

Fig. 1. Isolation scheme of the active compound present in *Atractylodes koreana*.

Identification of the active compound was analyzed by gas chromatography-mass spectrometry (GC-MS, Hewlett-Packard). The analytical condition employed was as follows: SPB-1 capillary column - 30 m×0.25 mm; Injection port temperature - 250℃; Column temperature started at 150℃ for 3 min, increased with 6℃/min, and maintained at 250℃ for 10 min. ^{1}H- and ^{13}C-NMR (Bruker AMX-500, Bruker Analytische Messtechnik Gmbh, Rheinstetten, Germany) analyses were also conducted.

Results

Insecticidal activity

Non-polar fractions of some medicinal plants possessed insecticidal potential effective to the diamondback moth larvae. Out of 168 polar or non-polar fractions obtained from 84 medicinal plants tested, only 32 non-polar fractions gave complete larval mortality of the diamondback moth at the respective application rate equivalent to 5000 ppm of the plant extracts (Chun & Han, 1998). However, none of the polar fractions showed the insecticidal activity.

Eleven non-polar fractions were selected from the secondary screening test (Table 1). Decrease in the larval mortality occurred as reducing the application rate of the non-polar fractions. The highest larval mortality at the lowest application rate was obtained with *A. koreana*. At 320 ppm, the fraction of *A. koreana* gave 70% larval mortality, whereas the rest showed only less than 35% mortality. Non-polar fractions of *P. arboricola*, *P. suffruticosa*, *A. villosum* and *S. china* also provided 100% larval mortality at 2500 ppm. When the application rate was further reduced to 1250 ppm, however, the mortality decreased in a different degree, depending on origin of the fractions. The results indicate that the fractions originated from the medicinal plants contain natural compounds with insecticidal potential against the diamondback moth larvae, but content and kind of the effective compounds seem to vary with the plant species.

Separation and Identification of the Active Compound from *Atractylodes koreana*

Non-polar fraction of 24.5 g prepared from *A. koreana* tubers was used to isolate and identify an active compound with insecticidal potential effective to the diamondback moth larvae (Fig. 1). After the fraction

Table 1. Mortality of diamondback moth as affected by non-polar fractions extracted from medicinal plants

Plant species	Mortality (% of control)					
	Concentration (ppm)					
	5000	2500	1250	630	320	160
Atractylodes koreana	100	100	100	100	70	0
Piper arboricola	100	100	95	10	5	0
Paeonia suffruticosa	100	100	60	15	0	0
Amomum villosum	100	100	60	5	0	0
Smilax china	100	100	25	10	5	0
Corydalis turtschaninowii	100	95	75	30	15	0
Morinda officinalis	100	80	45	40	25	0
Salvis miltiorrhiza	100	75	60	45	5	0
Rubus coreanus	100	75	55	45	30	0
Gledistia sinensis	100	60	55	50	15	0
Cyperus rotundus	100	50	50	45	35	0

Table 2. Larval mortality of diamondback moth by primary and secondary fractions obtained from non-polar crude extract of *Atractylodes koreana*

Fraction	Mortality (% of control)[a]						
Primary	F_1		F_2		F_3		F_4
	0		100		0		0
Scondary	F_{21}	F_{22}	F_{23}	F_{24}	F_{25}	F_{26}	F_{27}
	0	100	0	0	0	0	0

[a]Each fraction was bioassyed at 1,000 ppm.

was successively eluted in flash chromatography on silica gel with several organic solvents, four sub-fractions different in their polarity were obtained. Bioassay done at 1000 ppm of the respective sub-fractions revealed that ethyl ether fraction (F_2) provided a complete insecticidal activity, but there were no responses with n-hexane (F_1), ethyl acetate (F_3) and methanol (F_4) fractions (Table 2).

Further separation with F_2 fraction using preparative TLC resulted in confirmation of seven spots with different R_f values ranging from 0.59 to 0.06. Out of the fractions, only F_{22} spot with R_f value 0.45 was found to active against the diamondback moth larvae. There was no insecticidal activity with the rest of the spots.

The active compound obtained at R_f 0.45 was analyzed by GC-MS and ^{1}H- and ^{13}C-NMR analyses. Mass spectrum of the compound indicated molecular ion peak at *m/z* 218. Identification by ^{13}C-NMR also revealed that the compound showed 15 carbon signals with chemical shifts as indicated in Table 3. On the other hand, ^{1}H-NMR spectrum showed chemical shifts as follows (in ppm); 1.18 (3H, *s*, $-CH_3$), 1.75 (6H, *s*, $-CH_3$), 1.0-2.9 (10H, *m*, $-CH_2$), 2.48 (1H, *m*, >CH-), 4.76 (2H, *s*, $=CH_2$). From the spectral data, the compound was identified as 4,11-selinadien-3-one whose molecular formula was $C_{15}H_{22}O$ (Fig. 2).

Determination of 4,11-selinadien-3-one in other medicinal plants

As shown in Table 1, non-polar fractions of some medicinal plants besides *A. koreana* were also effective against the diamondback moth larvae. However, whether the effectiveness relied on 4,11-selinadien-3-one as in *A. koreana* is uncertain. Therefore, presence of this compound in the effective fractions was determined by TLC. When the fractions were developed along with 4,11-selinadien-3-one obtained from *A. koreana* as a standard, none of the fractions showed any spot with R_f value 0.45 of 4,11-selinadien-3-one. This result suggests that the fractions effective against diamondback moth do not contain 4,11-selinadien-3-one found in *A. koreana*.

Discussion

Plants produce hundreds of thousands of compounds as secondary metabolites. However, the functions of these compounds are largely unknown. Only few natural plant products have been exploited for potential use of pesticides. To investigate the pesticidal potential, plant materials are simply extracted by water or organic solvents and screened against agricultural pests. In the primary bioassay screening with the extracts of medicinal plants, some non-polar fractions showed an insecticidal activity against the diamondback moth larvae (Chun & Han, 1998). All the polar fractions, however, had no such activity. This finding indicates that active compounds with the insecticidal potential against the diamondback moth larvae present in the medicinal plants screened are generally hydrophobic in nature.

Eleven non-polar fractions selected based on the primary screening results were employed to determine concentration-dependent insecticidal activity (Table 1). All the fractions tested showed decrease in the mortality as reducing the application rates, but decrease in the mortality was not dependent upon dilution fold of the fractions. Among the fractions, the highest larval mortality to diamondback moth at 320 ppm of the respective plant fractions occurred with *A. koreana*. Variation in insecticidal activity of the fractions suggests that active compounds present in the medicinal plants may differ in their content and kind.

Non-polar fraction of *A. koreana* was selected to isolate and identify an active compound that provided effective larval mortality against the diamondback moth larvae. An active fraction occurred in ethyl ether fraction of counter-current distribution separation and in turn appeared at R_f 0.45 in preparative TLC analysis (Fig. 1, Table 2). When the active fraction was analyzed in GC-MS and ^{1}H- and ^{13}C-NMR, the compound was identified as 4,11-selinadien-3-one (Table 3, Fig. 2). This sesquiterpenoid compound is also called α-cyperone and has been synthesized by Haaksma *et al.* (1992).

Table 3. ^{13}C-NMR spectral data of 4,11-selinadien-3-one in $CDCl_3$

C	δ_c (Multi.)
1	37.42 (t)
2	33.78 (t)
3	199.10 (s)
4	128.79 (s)
5	162.15 (s)
6	32.90 (t)
7	45.83 (d)
8	26.89 (t)
9	41.90 (t)
10	35.81 (s)
11	149.12 (s)
12	20.67 (q)
13	109.20 (t)
14	22.47 (q)
15	10.91 (q)

Sesquiterpenic ketones in *Cyperus* species were first isolated and identified by Hikino *et al.* (1967). Recently, Dadang *et al.* (1996) determined potential insecticidal activity with the methanol extract of *C. rotundus* tubers against diamondback moth and isolated and identified α-cyperone Table 3. ^{13}C-NMR spectral data of 4,11-selinadien-3-one in $CDCl_3$ as the active compound.

Although the non-polar fraction of *C. rotundus* in our experiment showed insecticidal activity against the diamondback moth larvae in some degree (Table 1), however, there was no α-cyperone present in the plant material. This was confirmed by comparing R_f value of α-cyperone isolated from *A. koreana* as a standard with the fraction of *C. rotundus* (data not presented). The difference between the two results on *C. rotundus* is probably attributed to different sources of the plant materials. *C. rotundus* tubers Dadang *et al.* (1996) employed were collected from the tropical region, Bogor in Indonesia, whereas the plant material we used was originally cultivated in southern parts of Korea.

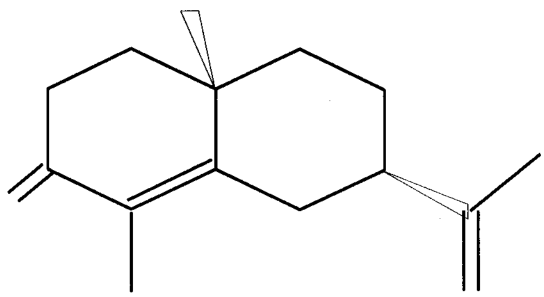

Fig. 2. Chemical structure of 4, 11-selinadien-3-oneisolated from *Atractylodes koreana*.

Atractylone and its derivatives are known as major pharmacological components in *Atractylodes* spp, while α-cyperone has not been found in the species (Hotta, 1989; Anon., 1996). In this experiment, however, we first isolated and identified α-cyperone from *A. koreana*. Since a sesquiterpenoid α-selinene (3,11-selinadiene) that is structurally similar to α-cyperone is present in *Atractylodes* spp., α-selinene might have been oxidized to produce α-cyperone under certain conditions in *A. koreana*.

Although function of α-cyperone in *A. koreana* and the tropical *C. rotundus* has not been disclosed yet, the results obtained in this experiment confirmed the insecticidal activity of α-cyperone against the diamondback moth larvae. The compound may be useful as lead for developing new novel insecticides. The ability to synthesize α-cyperone and its analogues has already been reported by Haaksma *et al.* (1992). Further investigation should focus on the detailed effects and the factors affecting its property.

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藥草 중에 存在하는 배추좀나방에 대한 天然殺蟲性 物質의 同定

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요약 : 약초 중에 존재하는 농약 활성 물질을 탐색하기 위하여 84종의 약초로부터 극성 및 비극성 분획을 얻고 주요 농업 해충에 대하여 살충성을 검토하였다. 일차 및 이차 검정 결과 참삽주 괴경 (창출)의 비극성 분획은 배추좀나방에 대하여 매우 높은 살충 효과를 보였다. 창출 비극성 분획내의 살충성 활성물질은 향류분배 분리, TLC, GC-MS, ^{1}H- 및 ^{13}C-NMR 분석으로 $C_{15}H_{22}O$의 4,11-selinadien-3-one (α-cyperone)으로 확인되었다. 그러나 배추좀나방에 대하여 높은 살충활성을 보였던 다른 비극성 분획으로부터는 α-cyperone을 검출할 수 없었다. 더욱이 α-cyperone이 향부자로부터 최초로 분리 동정 되었음에도 불구하고 우리 나라에서 재배된 향부자에서는 α-cyperone을 확인할 수 없었다.

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