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Feeding Deterrents from *Aconitum episcopale* Roots against the Red Flour Beetle, *Tribolium castaneum*

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ABSTRACT: The screening for insecticidal principles from several Chinese medicinal herbs showed that the ethanol extract of *Aconitum episcopale* roots possessed significant feeding deterrence against the red flour beetle, *Tribolium castaneum*. From the ethanol extract, six feeding deterrents were isolated by bioassay-guided fractionation. The compounds were identified as chasmanine, crassicauline A, karacoline, sachaconitine, talatisamine, and yunaconitine from their spectroscopic data. Chasmanine, talatisamine, karacoline, and sachaconitine exhibited feeding deterrent activity against *T. castaneum* adults, with EC_{50} values of 297.0, 342.8, 395.3, and 427.8 ppm, respectively. Yunaconitine and crassicauline A also possessed feeding deterrent activity against *T. castaneum* adults, with EC_{50} values of 653.4 and 1134.5 ppm, respectively.

KEYWORDS: Feeding deterrents, *Aconitum episcopale*, *Tribolium castaneum*, chasmanine, talatisamine

INTRODUCTION

Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for the design of target-specific molecules. During our screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, ethanol extracts of *Aconitum episcopale* Levl. (family: Ranunculaceae) roots were found to possess significant feeding deterrent activity against the red flour beetle, *Tribolium castaneum* Herbst. The red flour beetle is one of the most widespread and destructive primary insect pests of stored cereals.[1] Infestations not only cause significant losses because of the consumption of grains; they also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species.[2] *A. episcopale* was mainly found in 2200−3200 m high mountains of southwestern China (Guizhou, southwestern Sichuan, and Yunnan provinces) and is a beautiful climbing shrub that likes to clamber its way through, around, and over suitable supports and has been cultivated for gardens and roadsides because of its large and handsome, deeply cut foliage and clusters of exquisite blue monkshood flowers.[3] It is used as a folk medicine to treat fever, rheumatism, and fracture in Tibet and among the Naxi people of Lijiang, Yunnan province, China.[4] The very famous known chemical constituents of the genus *Aconitum* are their diterpenoid alkaloids. Several diterpenoid alkaloids have been isolated and identified from *A. episcopale* roots.[5−8] In the previous reports, some diterpenoid alkaloids have been demonstrated to possess insecticidal, feeding deterrent, and repellency activity.[9−13] However, the bioactive compounds against insects have not been isolated and identified from this Chinese medicinal herb. In this paper, we report the isolation and identification of six feeding deterrents contained in *A. episcopale* roots against *T. castaneum* (Figure 1).

MATERIALS AND METHODS

Extraction of Plant Material. *A. episcopale* (1.8 kg, dried roots), collected from Wuding County, Yunnan province, China, was ground to a powder and extracted with 95% ethanol (10 L) at room temperature over a period of 3 weeks. A voucher specimen was deposited in the museum of the Department of Entomology, China Agricultural University. The extracts were concentrated using a vacuum rotary evaporator to afford a syrupy gum (77 g). This syrup was partitioned between methanol−water and *n*-hexane (3 × 1000 mL). The *n*-hexane extracts were evaporated to given a residue (22 g). The aqueous layer was repartitioned with chloroform (3 × 1000 mL) to provide a residue (34 g) after evaporation of chloroform. Further partitioning with ethyl acetate (3 × 1000 mL) gave a residue (13 g) after evaporation of the solvent.

Apparatus. Melting points were measured on a Buchi 535. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance DRX 500 instruments using $CDCl_3$ as a solvent, with tetramethylsilane (TMS) as an internal standard. Electron impact mass spectrometry (EIMS) was determined on a ThermoQuest Trace 2000 mass spectrometer at 70 eV (probe).

Chromatography. The $CHCl_3$ residue (25 g) was applied to a silica gel column (160−200 mesh, Qingdao Marine Chemical Plant, Shandong province, China), eluting with petroleum ether, containing increasing accounts of ethyl acetate (from 100:1 to 1:2), to give 24 combined fractions according to thin-layer chromatography (TLC) detection. On the basis of the bioassay, fractions 3, 6, 8, 10, 12, 15, and 17 were chosen for further fractionation. Crassicauline A (**2**, 18.3 mg) was isolated from fraction 3 after being repeatedly purified on silica and parative thin-layer chromatography (PTLC) (precoated GF254

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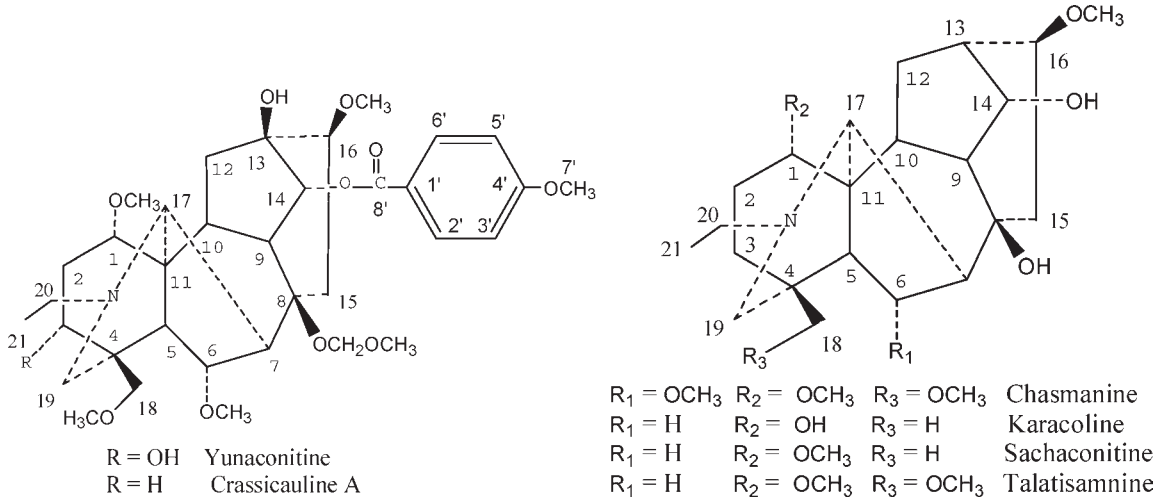

Figure 1. Structures of feeding deterrents isolated from *A. episcopale* roots.

plates, Qingdao Marine Chemical Plant). Fraction 8 was further chromatographed on a silica gel column and repeated PTLC to provide the bioactive compound, which was recrystallized and determined to be yunaconitine (**6**, 49.0 mg). Sachaconitine (**4**, 30.8 mg) was obtained from further chromatography on silica gel TLC and recrystallized from fraction 10. Fraction 12 was further chromatographed on a silica gel column and silica gel TLC to obtain talatisamine (**5**, 123.4 mg) after recrystallization. Karacoline (**3**, 7.9 mg) was obtained from further chromatography on silica gel TLC and recrystallized from fraction 15. Fraction 15 was further chromatographed on a silica gel column and silica gel TLC to give chasmanine (**1**, 59 mg). The structures of the compounds were elucidated on the basis of high-resolution EIMS and NMR.

Feeding Deterrence Bioassay. The red flour beetles, *T. castaneum*, were obtained from laboratory cultures maintained for the last 10 years in the dark in incubators at 28−30 °C and 70−80% relative humidity (RH). *T. castaneum* was reared on wheat flour mixed with yeast (10:1, w/w). Adult insects used in all of the experiment were about 2 weeks old. A commercial feeding deterrent, toosendanin (98%, from Chengdu Pusi Bio-Technology Company, Sichuan province, China) was used as a positive control. A flour disk bioassay was used to direct the isolation of active compounds from *A. episcopale* roots according to the method by Xie et al.,[14] with some modifications.[15] Wheat flour (0.8 g) was ultrasonically stirred in 4 mL of distilled water, and 50 μL of ethanol containing a fraction/compound was added. Pure compounds were first dissolved in 500 μL of ethanol, and 2 drops of Tween-20 (approximately 50 μg) were added to the wheat flour suspension. Aliquots of 200 μL of this stirred suspension were placed on the bottom of a polystyrene Petri dish to form disks. The pipet was fitted with a disposable tip that had an opening enlarged to about 2 mm in internal diameter by cutting about 1 cm from the bottom of the tip with a razor blade. The same amounts of ethanol and Tween-20 were applied to produce the control flour disks. The flour disks were left in the fume hood overnight to air dry. The flour disks were then transferred to an incubator to equilibrate at 28−30 °C and 70−80% RH for 48 h. Each flour disk weighed between 36 and 39 mg. The moisture content of the disk was determined to be 13.5 ± 0.1% using Kett's grain moisture tester (model PB-1D2, Japan). The disks were placed in glass vials (diameter, 2.5 cm; height, 5.5 cm) for weighing. A total of 20 group-weighed, unsexed insects were then added to each vial prior to further weighing. All of the insects were starved for 24 h before use. The experimental setup was left in the incubator for 3 days. Finally, insects and the uneaten parts of the flour disks were weighed. The insect consumption for the different test substances was compared to the control group. Glass vials containing treated flour disks but without insects were prepared to determine any decrease in weights that might have occurred because of evaporation of solvents. Five replicates of each concentration of the compounds and control were prepared for this experiment, including the controls without insects. All of the experiment set was repeated 3 times. Analysis of variance (ANOVA) and Tukey's test were conducted using SPSS 10 for Windows 98. The percentage was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests. The concentration needed to inhibit insect feeding by 50% relative to controls (EC_{50}) was determined by linear regression.

RESULTS AND DISCUSSION

Data for Isolated Bioactive Compounds. Chasmanine (**1**), white needles (acetone). mp 90−91 °C (90−92 °C[18]). EIMS (*m*/*z*): 451 [M^+] (4), 420 (100), 436 (10), 397 (90). $C_{25}H_{41}NO_6$. ^{1}H NMR (500 MHz, CDC1$_3$) δ: 4.63 (1H, s, −OH), 4.22 (1H, d, J = 6.8 Hz, H-6β), 3.74 (1H, d, J = 8.5 Hz, H-18β), 3.42 (1H, d, J = 8.5 Hz, H-18α), 3.36 (3H, s, −OCH_3), 3.33 (3H, s, −OCH_3), 3.32 (3H, s, −OCH_3), 3.26 (3H, s, −OCH_3), 3.16 (1H, s, H-17), 3.02 (1H, m, H-1), 1.08 (3H, t, J = 6.4 Hz, $NH_2\underline{CH_3}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 86.3 (C-1), 26.0 (C-2), 35.3 (C-3), 39.4 (C-4), 48.7 (C-5), 82.3 (C-6), 52.6 (C-7), 72.5 (C-8), 50.4 (C-9), 37.8 (C-10), 50.3 (C-11), 28.3 (C-12), 45.6 (C-13), 75.6 (C-14), 38.8 (C-15), 82.0 (C-16), 62.7 (C-17), 80.8 (C-18), 53.8 (C-19), 49.4 ($N\underline{CH_2}CH_3$), 13.8 ($NCH_2\underline{CH_3}$), 56.5 (1-OCH_3), 57.4 (6-OCH_3), 56.3 (16-OCH_3), 59.3 (18-OCH_3). The ^{1}H and ^{13}C NMR data were in agreement with the reported data.[18]

Crassicauline A (**2**), colorless needles (acetone). mp 163−165 °C (162.5−164.5 °C[6]). EIMS (*m*/*z*): 643 [M^+] (4), 612 (100), 584 (24), 583 (50), 568 (20), 552 (68), 432 (14), 400 (10), 252 (16), 251 (16), 135 (28), 60 (20), 45 (30), 43 (31). $C_{35}H_{49}NO_{10}$. ^{1}H NMR (500 MHz, CDC1$_3$) δ: 8.03 (2H, d, J = 8.7 Hz, H-2′, H-6′), 6.93 (2H, d, J = 8.8 Hz, H-3′, H-5′), 4.89 (1H, d, J = 5.0 Hz, H-14β), 3.98 (1H, d, J = 6.6 Hz, H-6β), 3.88 (3H, s, Ar-OCH_3), 3.54 (3H, s, −OCH_3), 3.30 (3H, s, −OCH_3), 3.28 (3H, s, −OCH_3), 3.17 (3H, s, −OCH_3), 1.34 (3H, s, −$OCOCH_3$), 1.11 (3H, t, J = 7.1 Hz, H-21). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 85.0 (C-1), 26.3 (C-2), 35.8 (C-3), 39.1 (C-4), 49.1 (C-5), 83.0 (C-6), 49.5 (C-7), 85.6 (C-8), 41.0 (C-9), 45.1 (C-10), 50.2 (C-11), 34.9 (C-12), 74.9 (C-13), 78.5

Table 1. Feeding Deterrent Activities of the Compounds Isolated from *Aconitum episcopale* Roots against *T. castaneum* Adults

compounds	concentration (ppm)	consumption of diet[a] (% control ± SD)	EC_{50} (95% FL)	slope ± SD	χ^2
control		100.00 ± 4.45 a			
chasmanine	1000	35.89 ± 4.93 f	297.0 (249.9−359.5)	1.91 ± 0.09	30.42
	300	51.23 ± 2.95 e			
	100	61.64 ± 3.21 d			
	30	72.47 ± 3.38 c			
	10	90.55 ± 3.79 b			
crassicauline A	1000	53.29 ± 3.75 d	1134.5 (894.5−1504.5)	2.59 ± 0.11	17.60
	300	69.59 ± 4.09 c			
	100	78.36 ± 3.83 c			
	30	89.73 ± 3.34 ab			
	10	97.81 ± 3.26 a			
karacoline	1000	39.59 ± 3.71 e	395.2 (326.8−489.9)	1.96 ± 0.09	16.59
	300	53.83 ± 3.64 d			
	100	65.07 ± 4.16 c			
	30	77.95 ± 3.96 b			
	10	92.96 ± 3.77 a			
sachaconitine	1000	43.84 ± 2.34 d	427.8 (311.4−635.9)	1.83 ± 0.13	50.94
	300	54.66 ± 3.87 c			
	100	62.19 ± 3.65 c			
	30	71.78 ± 4.34 b			
	10	93.97 ± 3.45 a			
talatisamine	1000	38.08 ± 2.89 d	342.8 (286.7−418.5)	1.96 ± 0.09	29.76
	300	53.70 ± 2.92 c			
	100	60.82 ± 2.83 c			
	30	75.89 ± 4.06 b			
	10	92.47 ± 3.28 a			
yunaconitine	1000	48.77 ± 2.33 e	654.3 (530.1−833.6)	2.24 ± 0.10	28.09
	300	58.63 ± 3.16 d			
	100	68.37 ± 2.84 c			
	30	85.48 ± 3.96 b			
	10	96.75 ± 3.34 a			
toosendanin			94.3 (86.5−102.7)		

[a] Multiple range test using Tukey's test ($p < 0.05$). The same letters denote treatments not significantly different from each other.

(C-14), 39.3 (C-15), 83.7 (C-16), 62.1 (C-17), 80.4 (C-18), 53.6 (C-19), 49.2 (C-20), 13.5 (C-21), 56.3 (1-OCH_3), 58.7 (6-OCH_3), 57.8 (16-OCH_3), 59.1 (18-OCH_3), 55.5 (Ar-OCH_3), 169.9 ($OCOCH_3$), 21.7 ($OCOCH_3$), 166.2 (ArCO−), 122.7 (C-1′), 131.7 (C-2′, C-6′), 113.7 (C-3′, C-5′), 163.4 (C-4′). The ^{1}H and ^{13}C NMR data were in agreement with the reported data.[16,17]

Karacoline (**3**), white crystal (acetone). mp 183−184 °C. EIMS (*m*/*z*): 377 [M^+],[19] 360 (100), 344 (34), 321 (56), 306 (31). $C_{22}H_{35}NO_4$. ^{1}H NMR (500 MHz, $CDC1_3$) δ: 4.25 (1H, t, J = 4.8 Hz, H-14β), 3.36 (3H, s, −OCH_3), 1.13 (3H, t, J = 7.1 Hz, NH_2CH_3), 0.90 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 72.5 (C-1), 29.7 (C-2), 31.4 (C-3), 32.9 (C-4), 46.7 (C-5), 25.1 (C-6), 46.6 (C-7), 74.2 (C-8), 45.1 (C-9), 44.1 (C-10), 48.8 (C-11), 28.2 (C-12), 39.8 (C-13), 75.9 (C-14), 42.3 (C-15), 81.9 (C-16), 63.5 (C-17), 27.6 (C-18), 60.2 (C-19), 48.5 (NCH_2CH_3), 13.1 (NCH_2CH_3), 56.4 (16-OCH_3). The ^{1}H and ^{13}C NMR data were in agreement with the reported data.[8,19]

Sachaconitine (**4**), colorless needles (ethyl acetate). mp 129−130 °C. EIMS (*m*/*z*): 391 [M^+] (20), 360 (100). $C_{23}H_{37}NO_4$. ^{1}H NMR (500 MHz, $CDC1_3$) δ: 4.84 (1H, d, J = 3.9 Hz, 14-OH), 4.16 (1H, q, J = 4.5 Hz, H-14β), 3.37 (3H, s, −OCH_3), 3.28 (3H, s, −OCH_3), 1.07 (3H, t, J = 7.0 Hz, NH_2CH_3), 0.80 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 86.7 (C-1), 26.3 (C-2), 37.6 (C-3), 34.7 (C-4), 50.9 (C-5), 25.2 (C-6), 45.8 (C-7), 73.0 (C-8), 47.1 (C-9), 38.0 (C-10), 49.0 (C-11), 27.8 (C-12), 45.8 (C-13), 75.7 (C-14), 38.5 (C-15), 82.3 (C-16), 62.6 (C-17), 26.3 (C-18), 56.9 (C-19), 49.5 (NCH_2CH_3), 13.8 (NCH_2CH_3), 56.4 (1-OCH_3), 56.5 (16-OCH_3). The ^{1}H and ^{13}C NMR data were in agreement with the reported data.[20]

Talatisamine (**5**), white crystal (ethyl acetate). mp 139−140 °C (138−140 °C[7]). EIMS (*m*/*z*): 421 [M^+] (5), 406 (3), 390 (100), 376 (2), 374 (2), 360 (2.5), 58 (3). $C_{24}H_{39}NO_5$. ^{1}H NMR (500 MHz, $CDC1_3$) δ: 4.86 (1H, s, −OH), 4.16 (1H, q, J = 4.6 Hz, H-14β), 3.66 (1H, s, −OH), 3.36 (3H, s, −OCH_3), 3.32 (3H, s, −OCH_3), 3.29 (3H, s, −OCH_3), 1.07 (3H, t, J = 6.8 Hz, H-21). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 86.4 (C-1), 25.9 (C-2), 32.8 (C-3), 38.7 (C-4), 46.1 (C-5), 24.8 (C-6), 45.8 (C-7), 72.9 (C-8), 47.0 (C-9), 45.9 (C-10), 48.7 (C-11), 27.7 (C-12), 37.5 (C-13), 75.6 (C-14), 38.3 (C-15), 82.3 (C-16), 63.0 (C-17), 79.5 (C-18), 53.2 (C-19), 49.6 (NCH_2CH_3), 13.7 (NCH_2CH_3), 56.4 (1-OCH_3), 56.5 (16-OCH_3), 59.5 (18-OCH_3). The ^{1}H and ^{13}C NMR data were in agreement with the reported data.[19,20]

Yunaconitine (**6**), colorless needles ($CHCl_3$/acetone). mp 143−144 °C (141−143 °C[5]). EIMS (*m*/*z*): 659 [M^+] (6), 628

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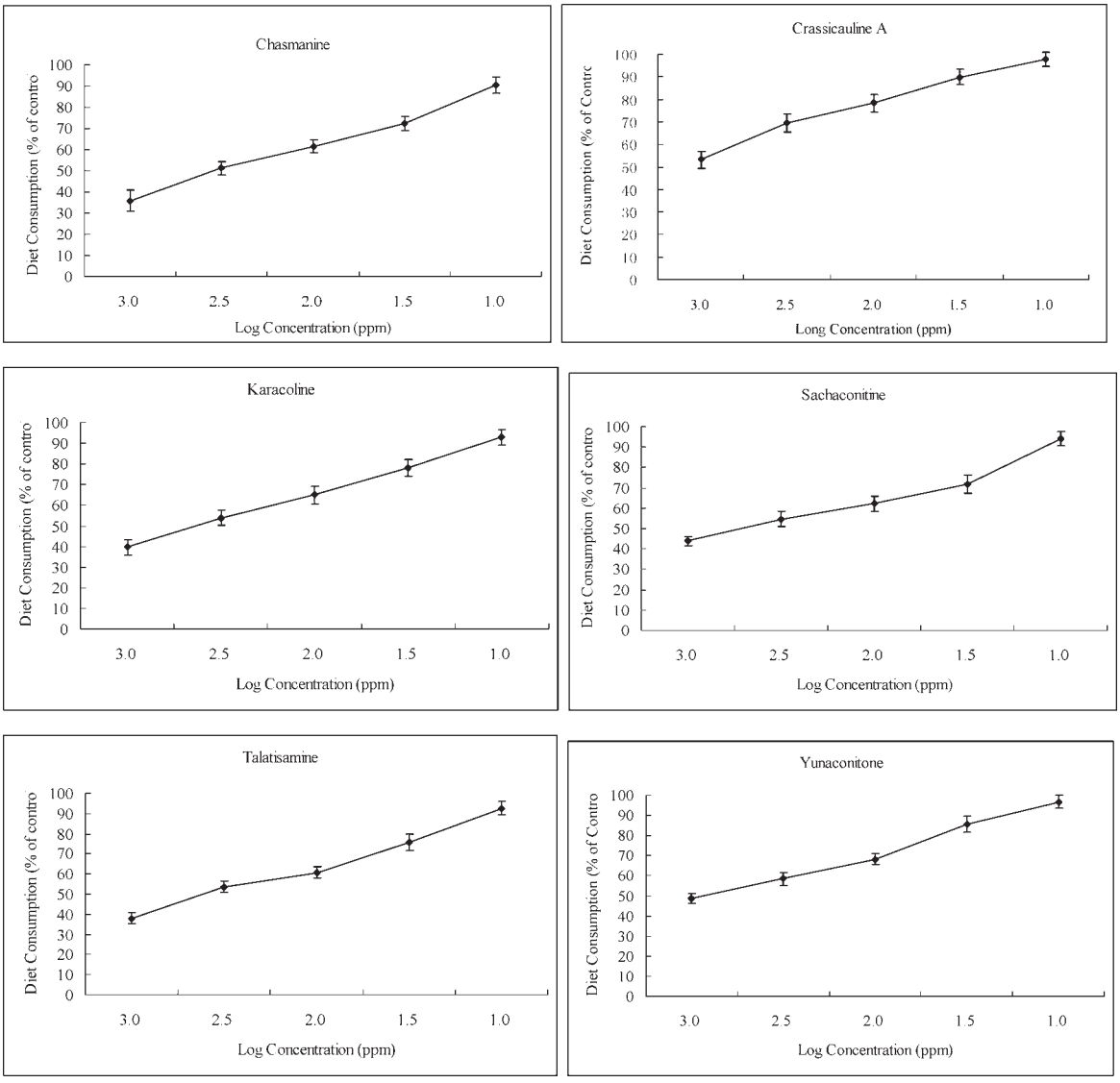

Figure 2. Dose−response curves of *T. castaneum* adults fed with diterpenoid alkaloid-treated diets.

(100), 599 (24), 585 (50), 569 (20), 135 (100). $C_{35}H_{49}NO_{11}$. ^{1}H NMR (500 MHz, $CDC1_3$) δ: 8.02 (2H, d, J = 8.7 Hz, H-2′, H-6′), 6.94 (2H, d, J = 8.7 Hz, H-3′, H-5′), 4.89 (1H, d, J = 5.1 Hz, H-14β), 4.05 (1H, d, J = 6.5 Hz, H-6β), 3.88 (3H, s, Ar-OCH_3), 3.56 (3H, s, −OCH_3), 3.31 (3H, s, −OCH_3), 3.27 (3H, s, −OCH_3), 3.18 (3H, s, −OCH_3), 1.36 (3H, s, −$OCOCH_3$), 1.12 (3H, t, J = 7.1 Hz, H-21). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 83.1 (C-1), 33.5 (C-2), 71.6 (C-3), 43.2 (C-4), 47.3 (C-5), 82.2 (C-6), 48.7 (C-7), 85.5 (C-8), 44.7 (C-9), 40.8 (C-10), 50.3 (C-11), 35.2 (C-12), 74.7 (C-13), 78.5 (C-14), 39.6 (C-15), 83.6 (C-16), 61.7 (C-17), 76.8 (C-18), 48.8 (C-19), 47.5 (NCH_2-CH_3), 13.3 (NCH_2CH_3), 55.9 (1-OCH_3), 58.9 (6-OCH_3), 57.8 (16-OCH_3), 59.2 (18-OCH_3), 55.5 (Ar-OCH_3), 169.9 (OCO-CH_3), 21.7 ($OCOCH_3$), 166.1 (ArCO), 122.6 (C-1′), 131.7 (C-2′, C-6′), 113.8 (C-3′, C-5′), 163.5 (C-4′). The ^{1}H and ^{13}C NMR data were in agreement with the reported data.[5,8]

The feeding deterrent activity of six isolated compounds and the positive control (toosendanin) against the red flour beetle was shown in Table 1. Incorporation of chasmanine into diets at concentrations of 10 ppm and above significantly (p < 0.05) reduced food consumption of *T. castaneum* adults compared to the control (Table 1). The consumption of diet (percentage of the control) at 10−1000 ppm of chasmanine ranged from 90.55 to 35.89%, and the EC_{50} value was calculated to be 297.0 ppm. The other five isolated compounds also significantly inhibited food consumption of *T. castaneum* adults at concentrations of 30 ppm and above in a concentration-dependent manner (Figure 2). Dietary talatisamine, karacoline, sachaconitine, yunaconitine, and crassicauline A also exhibited feeding deterrent activity, with EC_{50} values of 342.8, 395.2, 427.8, 653.4, and 1134.5 ppm, respectively (Table 1). In comparison to the commercial feeding deterrent, toosendanin, the six isolated compounds were 3−12 times less active against *T. castaneum* adults (EC_{50} value of toosendanin was determined as 94.3 ppm).

Feeding deterrents/antifeedants are gaining importance as potential components of integrated pest management strategies for insect control. There are numerous reports on the feeding deterrent, post-ingestive, and toxic effects as well as repellency of different classes of terpenoids/alkaloids against stored product insects.[15,21−25] Moreover, many diterpenoid alkaloids have been

demonstrated to possess insecticidal and feeding deterrent activity. For example, 43 norditerpenoid alkaloids were evaluated for insect feeding deterrent and toxic activity against Egyptian cottonworm, *Spodoptera littoralis*, and the Colorado potato beetle, *Leptinotarsa decemlineata*.[11] Acontine, hypacontine, and mesacontinine were found to possess post-ingestive and antifeedant activity against the beet armyworm, *Spodoptera exigua*,[12] and five diterpenoid alkaloids derived from the Chinese medicinal herb *Aconitum camichaeli* possessed insecticidal activity against the rice planthopper, *Nilaparvata lugens*, and alfalfa aphid, *Aphis medicaginis*, at a dose of 500 mg/L.[13] However, among all of the six isolated compounds, only karacoline was reported to have insecticidal and feeding deterrent activity against the Colorado potato beetle, *L. decemlineata*.[11] The other five compounds were evaluated for the first time for feeding deterrent activity against stored product insects.

When the feeding deterrent activity of the six compounds is compared, yunaconitine and crassicauline A were less active than the four other compounds to the red flour beetles (Table 1). It seems that the C-14 hydroxy group is important in the active molecules for *T. castaneum* (compounds **2**−**5**; Figure 1). Among the four compounds containing C-14 hydroxy group, chasmanine showed the strongest feeding deterrent activity against *T. castaneum* adults, while sachaconitine possessed the weakest activity (Table 1).

In traditional Chinese medicine, the tubers of *Aconitum* species have been extensively employed for the clinical treatment of pains, rheumatics, and neurological disorders. Some diterpenoid alkaloids, including crassicauline A and yunaconitine, have been reported to exhibit marked analgesic activities and have been developed to be analgesic drugs clinically used for the treatment of various pains in China.[26] However, many diterpenoid alkaloids are reported to possess high toxicity to mammals.[27,28] Isolated constituent yunaconitine was reported to have a mouse LD_{50} value of 2.97 mg/kg by oral injection, and another constituent karacoline was showed to exhibit a mouse LD_{50} value of 298 mg/kg by intraperitoneal injection and 51.5 mg/kg by intravenous injection.[29] However, no experimental data about the safety of extracts of this medicinal herb and the four other isolated constituents are available thus far. Therefore, any attempt to develop a diterpenoid alkaloid-derived agrochemical must be carefully evaluated for harmful effects.

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