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Abietane-type and labdane-type diterpenoids from the cones of *Chamaecyparis obtusa*

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Abstract A novel compound, 8(17),12E,14-labdatrien-19-al (*trans*-communal), was isolated from ethyl acetate extract of young cones of hinoki (*Chamaecyparis obtusa* Endl.). The chemical structure of the compound was determined mainly with various nuclear magnetic resonance spectral techniques. Its stereochemistry was determined by derivation to a known compound, *trans*-communol. In addition to this compound, four known compounds – ferruginol, chamaecydin, 12-hydroxy-6,7-seco-abieta-8,11,13-triene-6,7-dial, and *trans*-communic acid – were isolated. All isolated compounds were subjected to an antifeedant bioassay against the pest insect *Spodoptera litura*. Results of the bioassay showed that chamaecydin and 12-hydroxy-6,7-seco-abieta-8,11,13-triene-6,7-dial had antifeedant activity.

Key words *Chamaecyparis obtusa* · *Cupressaceae* · Antifeedant activity · *Spodoptera litura* · Diterpenoid

Introduction

Hinoki (*Chamaecyparis obtusa* Endl.), an evergreen widely distributed in Japan, is one of the most popular trees for timber use. Numerous chemical constituents have been isolated and identified from various parts of the tree.[1–6] Ozaki et al. reported a number of mono-, sesqui-, and diterpenes as constituents of hinoki seeds.[7] Hirose et al. also isolated unique quinone methide compounds (chamaecydin, isochamaecydin, chamaecydinol) from the seeds.[8] Although Shieh et al. investigated essential oils of hinoki cones and reported 62 monoterpenes and sesquiterpenes as constituents[9] and Asano and Yamamoto isolated two principal diterpenes of hinoki cones,[10] few studies about the chemical constituents of the cones, especially diterpines or higher terpenoid constituents, have been reported.

We report here on some diterpenes and a triterpene obtained from hinoki cones. One novel diterpene was isolated, and its chemical structure is discussed. In addition, the antifeedant activities of these compounds against the larvae of *Spodoptera litura* were studied.

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Experimental

Silica gel column chromatography was performed using Merck Kieselgel 60 (70–230 mesh). For thin-layer chromatography (TLC), Merck Kieselgel 60 CF_{254} was used. Flash chromatography was done with Eyela FC-10 equipped with an FCC-20 column loaded with 20 g of Merck Kieselgel 60 (230–400 mesh), with a benzene/EtOAc solvent system (with a changing ratio if necessary) as eluent. An Advantec SF-2120 fraction collector was used to fractionate the elute. Gas chromatography (GC) was performed using a Shimadzu GC-9A equipped with FID and a DB-5 capillary column (30 m × 0.25 mm).

All nuclear magnetic resonance (NMR) spectra were obtained using a Jeol Alpha 500. Each compound was dissolved in $CDCl_3$, and trimethylsilane (TMS) was used as an internal standard. Electron ionization mass spectrometry (EIMS) measurements were performed with a Jeol DX-303HF. High-resolution mass spectrometry (HRMS) and gas chromatography-mass spectrometry (GC-MS) were performed with a Hitachi M-80B. Infrared (IR) spectra were measured as a solution of CCl_4 with a Jasco IRA-2. The optical rotations of compounds were obtained using a Jasco DIP-140.

Isolation of compounds

Hinoki cones were harvested at the Kiso Experiment Station, Forestry and Forest Products Research Institute, Japan in August 1992. The cones (295.0 g, corresponding to 112.7 g of absolute dry weight) were crushed roughly with a food processor. They were immediately defatted with hexane using a Soxhlet apparatus to remove leaf wax and then subjected to extraction with EtOAc. The crude EtOAc extract (5.9 g), which was absorbed on 10 g of silica gel, was applied on a silica gel column (100 g, 2 × 40 cm), then eluted with benzene. The benzene eluate was repeatedly purified by flash chromatography and preparative TLC. GC and TLC (developed with benzene or 10% EtOAc in benzene) were used to monitor each fraction.

Ferruginol (**1**): 10.59 mg, mp 56°–57° (colorless prisms), $[\alpha]<^{D}_{25}>$ +79.3° (c = 0.3), ^{1}H NMR (500 MHz, $CDCl_3$); δ 0.91 (3H, s), 0.93 (3H, s), 1.16 (3H, s), 6.62 (1H, s, H-14), 6.82 (1H, s, H-11), 1.22 (3H, J = 6.6 Hz, isopropyl Me), 1.24 (3H, d, J = 6.6 Hz, isopropyl Me), 2.80 (1H, m H-15). EIMS 70 eV, *m/z* (rel.int): 286 $[M]^+$ 271, 243, 292, 201, 189, 175, 69. IR max cm^{-1}: 3650, 2950, 1500, 1480, 1420, 1160.

Chamaecydin (**2**): 11.16 mg, mp 196°–197° (yellow prisms), $[\alpha]^{D}_{25}$ +20.10° (c = 1), ^{1}H NMR (500 MHz, $CDCl_3$); δ 0.97 (3H, s), 0.99 (3H, s), 1.17 (3H, s), 1.29 (3H, d, J = 6.8 Hz), 1.31 (3H, d, J = 6.8 Hz), 3.18 (1H, septet, J = 6.8 Hz), 0.81 (3H, d, J = 6.8 Hz), 1.06 (3H, d, J = 6.8 Hz), 1.80 (1H, septet, J = 6.8 Hz), 1.05 (1H, dd, J = 8 and 4 Hz), 0.78 (1H, dd, J = 6 and 4 Hz), 0.72 (1H, ddd, J = 8, 6, 2 Hz), 7.74 (1H, OH). ^{13}C NMR (125.65 MHz, $CDCl_3$) δ 13.3 (C-24), 18.7 (C-2), 19.1 (C-20), 19.6 (C-6), 19.7 (C-29), 20.1 (C-16), 20.3 (C-30), 20.5 (C-17), 22.1 (C-19), 28.8 (C-28), 30.0 (C-26), 30.8 (C-27), 31.1 (C-18), 31.6 (C-15), 33.5 (C-23), 33.9 (C-4), 34.8 (C-25), 36.6 (C-1), 38.7 (C-10), 41.7 (C-3), 51.0 (C-5), 58.0 (C-22), 123.6 (C-13), 136.4 (C-7), 144.7 (C-9), 145.1 (C-8), 149.4 (C-14), 151.8 (C-11), 182.6 (C-12), 205.7 (C-21). EIMS 70 eV, *m/z* (rel.int): 448 $[M]^+$, 405, 366, 314, 302, 287, 136, 121, 93. IR max cm^{-1}: 3290, 2950, 1700, 1615, 1430.

12-Hydroxy-6,7-seco-abieta-8,11,13-triene-6,7-dial (**3**): 5.97 mg, mp 181°–182° (colorless needles), $[\alpha]^{D}_{25}$ +10.4° (c = 1), ^{1}H NMR (500 MHz, $CDCl_3$); δ 0.72 (3H, s, H-19), 1.02 (3H, s, H-18), 1.27 (3H, d, J = 6.7 Hz, H-16), 1.28 (3H, d, J = 6.7 Hz, H-17), 1.51 (3H, s, H-20), 1.5–1.6 (2H, m, H-2), 1.7 (1H, m, H-1), 2.3 (1H, m, H-1), 3.13 (1H, d, J = 3.6 Hz, H-5), 3.20 (1H, q, J = 6.7 Hz, H-15), 5.67 (1H, s, −OH), 6.94 (1H, s, H-11), 7.84 (1H, s, H-14), 9.86 (1H, d, J = 3.6 Hz, H-6), 10.49 (1H, s, H-7). ^{13}C NMR (125.65 MHz, $CDCl_3$) δ 19.3 (C-3), 22.2 (C-16 and C-17), 26.7 (C-15), 27.3 (C-19), 28.0 (C-20), 30.1 (C-18), 33.5 (C-4), 37.4 (C-1), 37.6 (C-2), 40.5 (C-10), 64.6 (C-5), 114.8 (C-11), 128.2 (C-8), 132.2 (C-13), 134.5 (C-14), 151.1 (C-9), 157.5 (C-12), 191.4 (C-7), 205.8 (C-6). EIMS 70 eV, *m/z* (rel.int): 316 $[M]^+$ (68), 301 $[M-Me]^+$ (54), 287 $[M-CHO]^+$ (59), 273 $[M-43]^+$(34), 231 (47), 217 (37), 203 (100). IR max cm^{-1}: 3300, 2950, 1720, 1500, 1680, 1570, 1160, 910.

8(17),12E,14-Labdatrien-19-al (**4**): 4.96 mg, mp 131°–133° (oily substances), $[\alpha]^{D}_{25}$ +11.5° (c = 1), ^{1}H NMR (500 Mz, $CDCl_3$); δ 0.62 (3H, s, H-20), 1.03 (3H, s, H-18), 1.15 (1H, m, H-6), 1.50 (1H, m, H-5), 1.55–1.60 (2H, m, H-2), 1.75 (3H, s, H-16), 1.80 (1H, m, H-9), 1.85 (1H, m, H-6), 2.0 (1H, m, H-1), 2.03 (2H, m, H-7), 2.15 (2H, m, H-3 and H-11), 2.4 (1H, m, H-11), 2.45 (1H, m, H-1), 4.50 (1H, s, H-17), 4.88 (1H, d, J = 10.7 Hz, H-15), 4.89 (1H, s, H-17), 5.05 (1H, d, J = 17.7 Hz, H-15), 5.40 (1H, t, J = 6.4 Hz, H-12), 6.33 (1H, dd, J = 10.7 and 17.7 Hz, H-14), 9.76 (1H, s, H-19). ^{13}C NMR (125.65 MHz, $CDCl_3$); δ 11.8 (C-16), 13.6 (C-20), 19.3 (C-2), 23.4 (C-11), 23.8 (C-7), 24.4 (C-18), 34.4 (C-3), 38.2 (C-1), 38.6 (C-6), 39.9 (C-10), 48.6 (C-4), 55.8 (C-9), 56.0 (C-5), 108.5 (C-17), 110.0 (C-15), 133.5 (C-13), 133.6 (C-12), 141.5 (C-14), 147.3 (C-8), 205.6 (C-19). GC-MS 70 eV, *m/z* (rel.int): 286 (7), 243 (6), 201 (9), 187 (9), 163 (10), 147 (10), 135 (18), 119 (19), 107 (34), 91 (39), 81 (72), 41 (100). HRMS for $C_{20}H_{30}O$ requires: 286.2295; found: 286.2322. IR max cm^{-1}: 2950, 1720, 1640, 1600, and 900.

trans-Communic acid (**5**): 10.03 mg, mp 125°–127° (colorless plate), $[\alpha]^{D}_{25}$ +34.6° (c = 1), ^{1}H NMR (500 MHz, $CDCl_3$); δ 0.65 (3H, s, H-20), 1.08 (1H, m, H-3), 1.15 (1H, m, H-6), 1.25 (3H, s, H-18), 1.36 (1H, m, H-9), 1.55 (1H, m, H-2), 1.75 (3H, s, H-16), 1.75 (1H, m, H-5), 1.89 (1H, m, H-2), 1.90 (2H, m, H-6 and H-7), 1.95 (1H, m, H-1), 2.00 (1H, m, H-7), 2.15 (1H, m, H-11), 2.18 (1H, m, H-3), 2.40 (2H, m, H-1 and H-11), 4.47 (1H, s, H-17), 4.84 (1H, s, H-17), 4.88 (1H, d, J = 11.0 Hz, H-15), 5.04 (1H, d, J = 17.5 Hz, H-15), 5.41 (1H, t, J = 6.0 Hz, H-12), 6.33 (1H, dd, J = 11.0 and 17.5 Hz, H-14). ^{13}C NMR (125.65 MHz, $CDCl_3$); δ 11.8 (C-16), 12.8 (C-20), 19.9 (C-2), 23.3 (C-11), 25.8 (C-7), 29.0 (C-18), 37.9 (C-3), 38.5 (C-1), 39.2 (C-6), 40.4 (C-10), 44.2 (C-4), 56.2 (C-9), 56.4 (C-5), 107.7 (C-17), 109.9 (C-15), 133.4 (C-13), 133.9 (C-12), 141.6 (C-14), 147.9 (C-8), 183.9 (C-19). EIMS 70 eV, *m/z* (rel.int): 302$[M]^+$ (93), 287 $[M-Me]^+$ (49), 257 $[M-COOH]^+$ (22), 246 (25), 222 (27), 204 (30), 175 (65), 161 (47), 147 (68), 135 (78), 119 (100). IR max cm^{-1}: 3300–2600, 2950, 1695, 1500, 1270, 905, and 895.

Bioassay

For the antifeedant bioassay, cellulose nitrate membrane filters (0.45 mm thickness, 13 mm diameter) were used as substrate. A 20-ml aliquot of diethyl ether solution of the isolated compound was soaked into a filter. Each compound was subjected to bioassay in 1000, 100, and 10 ppm solutions, and the treated discs contained 20, 2.0, and 0.2 μg of compound, respectively. For the control, the same amount of diethyl ether was soaked into another filter. After the solvent was evaporated for a minute at room temperature, the filters were pierced by pushpins settled upside down to make about 4 mm of space between filter and floor. A pair of substrates, sample and control, were put in a plastic petri dish (5 cm diameter) about 1 cm apart. The supernatant of a water-suspended artificial diet (1 g/10 ml) was soaked into each substrate as feeding stimulant. Three fourth-instar larvae of the common cutworm *Spodoptera litura* starved for 4 h were placed in each petri dish, which were then kept under dark conditions at 24°C for 2 h. Five replicates were prepared for each sample. The membrane filters fed by the larvae were removed from the pushpins, and their remain-

ing areas were measured with a Macintosh computer system containing NHI Image software and equipped with a 300-dpi image scanner.

Results and discussion

Components of the cones

Following purification of EtOAc extract of cones using silica gel columns, the five compounds shown in Fig. 1 (compounds **1–5**) were isolated from benzene eluate. Compound **1** (0.0094%, based on the oven-dried weight of seeds) gave an $[M]^+$ peak at *m/z* 286 by EIMS. An absorption band at 3650 cm^{-1} in the IR spectrum suggested the presence of a free hydroxy group. The ^1H NMR spectrum showed a couple of singlet signals at δ 6.62 and 6.82, which were attributed to aromatic protons. In addition, signals at δ 1.22 (3H, d, J = 6.6 Hz), 1.24 (3H, d, J = 6.6 Hz), and 2.80 (1H, m) suggested that isopropyl was a moiety attached to an aromatic ring in the structure. A comparison of spectral data with the literature[11] indicated compound **1** to be ferruginol.

Compound **2** (0.0099%, based on the oven-dried weight of seeds) gave an $[M]^+$ peak at *m/z* 448. An absorption band at 3290 cm^{-1} in the IR spectrum and a singlet signal at δ 7.74 in the ^1H NMR spectrum suggested the presence of a hydroxy group attached to a double bond. Except for the signal of the hydroxy proton, no signal was observed in a magnetic field lower than δ 3.50 ppm. The ^{13}C NMR spectrum showed two signals that arose from carbonyl carbons at δ 182.6 and 205.8. In addition, distortionless enhancement by polarization transfer (DEPT) spectral analyses revealed the presence of seven methyl carbons. Because NMR spectral data, especially all the ^{13}C NMR signals, were completely matched in the literature,[8] compound **2** was determined to be chamaecydin.

The EIMS data of compound **3** (0.0053%, based on oven-dried weight of seeds) showed an $[M]^+$ peak at *m/z* 316. The presence of a fragment ion peak at *m/z* 287 (M-29) suggested that the compound had an aldehyde group in its structure. Following the measurement of ^1H and ^{13}C NMR spectra, heteronuclear single quantum coherence (HSQC) spectral analyses were conducted, which assigned all protons to carbons. The ^1H NMR spectrum showed characteristic signals of a phenolic hydroxy proton at δ 5.67, an aliphatic aldehyde proton, which coupled with a CH proton at δ 3.13 (J = 3.6 Hz) at δ 9.86 and an aromatic aldehyde proton at δ 10.49. The chemical shift of CH proton at C-15 (δ 3.20) suggested that the isopropyl group would connect to the aromatic ring. Long-range ^1H-^{13}C couplings observed by heteronuclear multiple-bond correlation (HMBC) spectral analyses showed that the proton at C-11 had long-range correlations with C-8, C-10, and C-13; and the proton at C-14 had correlations with C-7, C-9, C-12, and C-15. Additionally, connections among CH_2 carbons (C-1, C-2, C-3) belonging to the aliphatic ring moiety were clarified by the COSY spectrum. Finally, compound **3** was determined to be 12-hydroxy-6,7-seco-abieta-8,11,13-triene-6,7-dial. In addition, the positive $[\alpha]_{25}^{D}$ +10.4° that compound **3** showed confirmed that the stereochemistry of **3** was the same as that shown in the literature.[12]

Compound **4** (0.0044%, based on the oven-dried weight of seeds) showed an $[M]^+$ peak at *m/z* 286 in its EIMS spectrum, and HRMS (*m/z* 286.2322) indicated that the molecular formula of **4** should be $C_{20}H_{30}O$. A strong absorption band at 1720 cm^{-1} in the IR spectrum and a singlet signal at δ 9.76 in the ^1H NMR spectrum suggested the presence of an aldehyde group. The ^{13}C NMR spectrum showed six double bond carbon signals (δ 108.5, 110.0, 133.5, 133.6, 141.5, 147.3), and the DEPT spectrum made it clear that the signals were from an exomethylene moiety and a terminal vinyl moiety.

In addition, three CH_3 carbon signals were observed at δ 11.8, 13.6, and 24.4 ppm. Following assignment of all protons to carbons by the HSQC spectrum, a tentative structure of compound **4** was presumed to be 8(17),12,14-labdatrien-19-al (communal) based on the HMBC spectrum shown in Fig. 2a. The nuclear Overhauser effect (NOE) was observed between protons at C-12 and C-14 (Fig. 2b), suggesting that the geometrical conformation of the double bond between C-12 and C-13 would be *trans*. Moreover, the ^1H NMR spectral data of 8(17),12Z,14-labdatrien-19-al (*cis*-communal) in the literature[13] was not matched with that of **4**. To determine the structure, compound **4** was subjected to reduction with $LiAlH_4$ in ether. It was confirmed that the reduction product (**4′**) was *trans*-communol by comparing the ^1H NMR spectral data with information in the literature.[14,15] Because **4′** showed positive specific rotatory power ($[\alpha]_{25}^{D}$ +11.3°, it was apparent that the stereochemistry of compound **4** was the same as (+)-*trans*-communol (**4′**). Thus, compound **4** was identified as 8(17),12E,14-labdatrien-19-al (*trans*-communal).

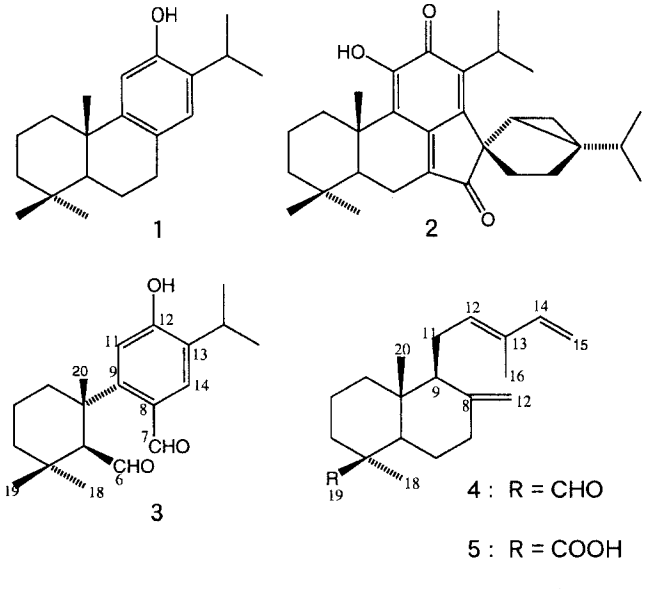

Fig. 1. Compounds isolated from cones of *Chamaecyparis obtusa*

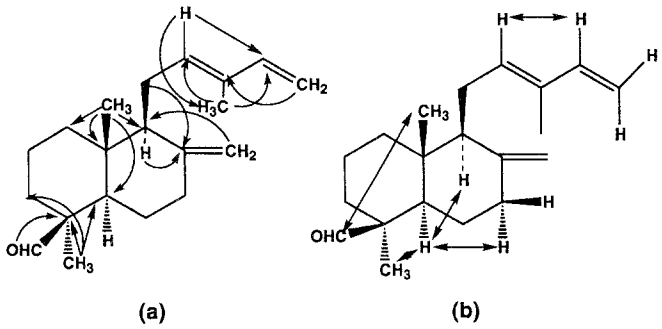

Fig. 2. Selected HMBC (**a**) and NOESY (**b**) correlations for **4**

Compound **5** (0.0089%, based on the oven-dried weight of seeds) gave an $[M]^+$ peak at *m/z* 302. Absorption bands at 3290 and 1700 cm^{-1} in the IR spectrum suggested the presence of a carboxylic acid group. The ^1H and ^{13}C NMR spectra of **5** showed signal patterns similar to those of compound **4** except for a signal due to a carbon of the carboxylic acid group substituted for the aldehyde group. In addition, the fact that reduction of **5** with $LiAlH_4$ also gave *trans*-communol (**4'**) indicated that **5** would be *trans*-communic acid.

Compound **4** identified as a *trans*-communal was not reported from nature until now. On the other hand, *cis*-communal, the geometrical isomer of **4**, was reported only as a constituent of *Juniperus communis* berries.[13] Two labdane-type diterpenoids, compounds **4** and **5**, were not reported as constituents of *Chamaecyparis obtusa*. Compounds **1**, **2**, and **3** have already been reported as constituents of hinoki seeds.[7,8] Because immature seeds and other tissues of cones were not distinguished when they were extracted during this research, it was expected that some constituents found in seeds would be isolated from the extract.

Antifeedant bioassay of the components for *Spodoptera litura*

The results of the bioassay are summarized in Table 1. The consumed amounts of disks were evaluated as square millimeters by measuring the remaining area. For convenience when comparing activities, the antifeedant index (AFI) $[(C - T)/(C + T)]$ (where C is the consumed area of control, and T is the consumed area of treatment disk) was also calculated.[16] Values in for the AFI ranged from 1, a potent antifeedant, to −1, a phagostimulant.

Chamaecydin (**2**) showed significant antifeedant activity at 20 μg/disk (AFI 0.44; 32.4 ± 9.1 mm^2 of the treated disk was consumed versus 83.5 ± 18.7 mm^2 of the control disk). 12-Hydroxy-6,7-seco-abieta-8,11,13-triene-6,7-dial (**3**) also showed activity at the same dose (AFI 0.78; 7.6 ± 4.6 mm^2 of the treated disk was consumed versus 61.2 ± 20.7 mm^2 of the control disk). Both compounds showed no significant activity at 2.0 and 0.2 μg/disk. The AFI of *trans*-communic acid (**5**) suggested possible antifeedant activity at 20 μg/disk (AFI 0.71; 2.2 ± 0.6 mm^2 of the treated disk was consumed versus 13.0 ± 4.5 mm^2 of the control disk) though no significant activity was detected. Interestingly, compound **5** reversed its activity at 2 μg/disk and showed some preference though it was not significant (AFI −0.63). The reason for this change is ambiguous and under investigation. Other compound showed no significant activity.

Table 1. Results of antifeedant bioassay against *Spodoptera litura*

Treatment (μg/disk)	Consumed area (mm^2) (mean ± SE)		Antifeedant index (C − T)/(C + T)
	Treated (T)	Control (C)	
Compound **1**			
20.0	9.8 ± 5.6	15.6 ± 5.7	0.23
2.0	13.0 ± 7.7	10.8 ± 4.2	−0.09
0.2	9.9 ± 4.2	4.4 ± 3.0	−0.38
Compound **2**			
20.0	32.4 ± 9.1*	83.5 ± 18.7	0.44
2.0	39.5 ± 16.4	61.3 ± 14.4	0.22
0.2	42.5 ± 17.8	62.7 ± 16.2	0.19
Compound **3**			
20.0	7.6 ± 4.6*	61.2 ± 20.7	0.78
2.0	7.0 ± 1.8	10.2 ± 5.3	0.19
0.2	23.0 ± 13.8	39.1 ± 8.3	0.26
Compound **4**			
20.0	29.7 ± 20.5	33.1 ± 10.4	0.06
2.0	5.8 ± 2.5	4.3 ± 1.8	−0.15
0.2	7.1 ± 2.4	3.9 ± 2.0	−0.29
Compound **5**			
20.0	2.2 ± 0.6	13.0 ± 4.5	0.71
2.0	15.7 ± 7.4	3.5 ± 1.9	−0.63
0.2	6.6 ± 3.3	8.1 ± 6.7	0.10

*Significant activity, $P < 0.05$

Lajide et al.[17] reported that aristolochic acid had strong antifeedant activity against *Spodoptera litura* (Fabricius) at 100 ppm. They also reported moderate activity of 6-hydroxyaristolochic acid at 1000 ppm. Although our results could not be compared directly with theirs because of differences in the bioassay method, compounds **2** and **3** could be appraised as moderate antifeedants against *S. litura*, similar to 6-hydroxyaristolochic acid.

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