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Bioactivity of Latifolin and Its Derivatives against Termites and Fungi

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Latifolin (**1**) and its derivatives were investigated with the aim of confirming the correlation between bioactivity (antitermite and antifungal activity) and chemical structure. Termite mortality in response to the derivatives 2′-*O*-methyllatifolin (**2**), latifolin dimethyl ether (**4**), and latifolin diacetate (**5**) increased 2-fold compared to compound **1**. The mortality rate from 5-*O*-methyllatifolin (**3**) was not different from **1**. The mass loss (feed consumption by termite) in response to compounds **3**−**5** was 3 times greater than compound **1**, and the mass loss from compound **2** was twice as great as compound **1**. The mortality rate from compounds **4** and **5** increased sharply 7 days after initial exposure. In assessing the antifungal activity of these compounds, it was found that the inhibition rates of white- and brown-rot fungi in response to all derivatives were less than that for compound **1**. Our findings indicate that the phenolic hydroxyl group at C-5 of the A ring provides antitermite activities (mortality and mass loss). In addition, both C-5 and C-2′ phenolic hydroxyl groups in the A and B rings have antifungal activity against white- and brown-rot fungi. In conclusion, the bioactivity of compound **1** depends upon the position of phenolic hydroxyl groups.

KEYWORDS: Latifolin; neoflavonoid; derivative; bioactivity; antitermite activity; antifungal activity

INTRODUCTION

Flavonoids are a group of compounds that have a phenylchromone structure (C6−C3−C6), and some of them exist as glycosides. They are classified as flavonoids, isoflavonoids, neoflavonoids, flavanonols, flavanones, isoflavones, or anthocyanidins (*1*).

It is well-known that *Fagus* and *Acacia* trees contain flavonoids and isoflavonoids, while *Macherium* and *Dalbergia* trees contain isoflavonoids and neoflavonoids (*1*). There are fewer plants that contain neoflavonoids than those that contain flavonoid and isoflavonoid, and probably for that reason, neoflavonoids have been less studied than flavonoids and isoflavonoids.

Most of neoflavonoids possess a 4-phenylchromone skeleton (*1*) and are classified by open or closed C rings. It has been reported widely that the closed type of neoflavonoids are found in Leguiminosae, Passifloraceae, Rubiaceae, Guttiferae, and Compositae (*1*). For example, kuhlmannin (*2*) and mesuol (*3*) are closed types of neoflavonoids, and bioactivities of them were reported, along with antioxidative activities (*2*), inhibition of HIV-1 replication,(*3*) etc.

On the other hand, only a few reports have identified the open type of neoflavonoids in *Dalbergia* and *Machaerium* of Leguiminosae and *Calophyllum* of Guttiferae (*1*). Of the open type of neoflavonoids, some dalbergiones (*4*, *5*) have been found to act as dermatitis agents. However, the biological function of neoflavonoids of the open type has been investigated to a lesser extent than that of closed types of neoflavonoids, flavonoids, and isoflavonoids.

In the genus *Dalbergia*, approximately 10 species, including *D. melanoxylon* and *D. nigra*, are used as wood products. *D. latifolia*, which is found in India and Indonesia, is a highly regarded fancy wood.

From the wood of *D. latifolia*, the open type of neoflavonoid molecules latifolin (**1**) (*6*), 4-methoxydalbergione (*7*−*9*), and obtusaquinol (*10*) have been identified. This wood is quite durable and is reported to have termite antifeedant and antifungal activity because of various extractives (*10*, *11*).

Recently, 4-methoxydalbergione, dalbergiphenol, and compound **1**, all of which are classified as open-type neoflavonoids, were isolated from the *n*-hexane extract of *D. latifolia* heatwood and were identified as bioactive molecules (*12*). Of the three isolated compounds, the bioactivity level of compound **1** was found to be the greatest against *Reticulitermes speratus* Kolbe and wood-decay fungi (*Trametes versicolor* and *Fomitopusis palustris*). These findings suggested that the bioactivity of neoflavonoids is affected by the presence of phenolic hydroxyl and methoxy groups.

Therefore, in this study, open-type neoflavonoids were isolated from durable wood, such as *Dalbergia* and *Machaerium*, which compound **1** and its derivatives were investigated, with the aim of confirming the correlation between bioactivity and chemical structure.

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MATERIALS AND METHODS

Preparation of Compound 1 and Its Derivatives. *Identification of Compound 1 and Its Derivatives*. The identification of compound **1** and its derivatives was conducted by spectral analysis, gas−liquid chromatography (GLC)/mass spectroscopy (MS), nuclear magnetic resonance (NMR), and optical rotation. GLC analysis was conducted with a SHIMADZU QP-5000 GLC/MS system, using a DB-1 capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA). The column temperature was from 150 °C (1 min) to 320 °C (5 min), heated at a rate of 5 °C/min. The injector temperature was 230 °C; the detector temperature was 250 °C; and the acquisition mass range was 50−450 amu using helium as a carrier gas (1.7 mL/min). One-dimensional (1D) and two-dimensional (2D) NMR were performed by a JEOL JNM-EX400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. Optical rotation of compound **1** was determined by a Horiba SEPA-300 polarimeter. The chemical structures of compounds **1**−**5** are shown in **Figure 1**.

Preparation of Compound 1. Compound **1**, isolated from *D. latifolia* wood in previous work (*12*), was used in this research.

R-(−)-latifolin (1). MS, *m*/*z* (relative intensity): 286 $[M]^+$ (47), 269 (4), 255 (25), 240 (3), 227 (3), 211 (4), 193 (2), 180 (9), 167 (12), 154 (100), 139 (16), 133 (13), 131 (13), 115 (10), 107 (12), 105 (6), 91 (7), 77 (13), 69 (15), 65 (7), 51 (8). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 3.84 (3H, s, $-OCH_3$), 3.86 (3H, s, $-OCH_3$), 5.04 (1H, ddd, $=CH_{2\ trans}$, J = 16.55, 1.51, 1.51), 5.18 (1H, ddd, C−H_A, J=5.86, 1.74, 1.74), 5.26 (1H, ddd, $=CH_{2\ cis}$, J = 10.31, 1.40, 1.40), 6.32 (1H, ddd, C−H_X, J = 17.09, 10.35, 5.95), 6.51 (1H, s, C3−H), 6.74 (1H, s, C6−H), 6.80−7.25 (4H, m, B ring). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 40.11 (C−H_A), 56.17 (2-OCH_3), 57.19 (4-OCH_3), 97.21 (C3), 115.33 (C6), 116.30 (C3′), 116.67 ($=CH_2$), 120.61 (C5′), 122.74 (C1), 127.71 (C6′), 128.53 (C4′), 129.42 (C1′), 139.09 (C−H_X), 140.15 (C5), 145.60 (C4), 149.55 (C2), 153.79 (C2′). $[\alpha]^{21.8}_D$ −27.12 (*c* 0.5, MeOH) (*13*).

Methylation. A total of 50 mg of compound **1** (*12*) was added to an ether solution of diazomethane prepared by reacting KOH in EtOH with *p*-tolylsulfonylmethylnitrosoamide in ether. The methylation was stopped before proceeding completely to give partial methylated products. Methylated compound **1** was isolated using column chromatography. The absorbent used was silica gel (60 N, spherical 63−210 μm, neutral; Kanto Chemical Co., Inc., Japan), and the column was eluted with a gradient of *n*-hexane−EtOAc to obtain derivatives **4**, **2**, and **3**.

Latifolin was methylated with diazomethane, and the products were analyzed by GC/MS. Three compounds were detected by the gas chromatograph. The molecular weight of these compounds was analyzed, and the purity was checked. One of the compounds was identified as latifolin dimethyl ether (**4**). On the basis of 1D NMR (^{1}H and ^{13}C) and NOESY−NMR (**Figure 2**), the other compounds were identified as 2′-*O*-methyllatifolin (**2**) and 5-*O*-methylatifolin (**3**), respectively.

2-O-Methyllatifolin (2). MS, *m*/*z* (relative intensity): 300 $[M]^+$ (100), 285 (8), 271 (16), 253 (8), 237 (7), 225 (8), 209 (8), 192 (19), 177 (16), 167 (25), 163 (24), 161 (22), 137 (38), 121 (42), 115 (41), 107 (17), 105 (17), 91 (64), 77 (37), 69 (51), 58 (42). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 3.65 (3H, s, $-OCH_3$), 3.69 (3H, s, $-OCH_3$), 3.80 (3H, s, $-OCH_3$), 4.70 (1H, ddd, $=CH_{2\ trans}$, J = 17.08, 1.71, 1.71), 5.07 (1H, ddd, $=CH_{2\ cis}$, J = 10.18, 1.59, 1.59), 5.36 (1H, brd, C−H_A, J = 5.92), 6.13 (1H, ddd, C−H_X, J = 17.20, 10.41, 5.54), 6.44 (1H, s, C3−H), 6.60 (1H, s, C6−H), 6.77−7.13 (4H, m, B ring). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 41.56 (C−H_A), 57.16 ($-OCH_3$), 57.53 ($-OCH_3$), 58.85 ($-OCH_3$), 99.32 (C3), 111.52 (C1), 112.27 (C3′) 116.75 (C6), 116.93 ($=CH_2$), 119.17 (C1′), 121.72 (C5′), 128.69 (C4′), 130.75 (C6′), 140.64 (C5), 141.64 (C−H_X), 146.36 (C4), 152.27 (C2), 158.50 (C2′).

5-O-Methyllatifolin (3). MS, *m*/*z* (relative intensity): 300 $[M]^+$ (27), 285 (1), 269 (11), 253 (2), 241 (2), 225 (3), 210 (2), 194 (5), 181 (12), 168 (100), 153 (32), 131 (14), 115 (8), 107 (13), 91 (9), 77 (15), 69 (19), 51 (8). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 3.74 (3H, s, $-OCH_3$), 3.84 (3H, s, $-OCH_3$), 3.85 (3H, s, $-OCH_3$), 5.01 (1H, ddd, $=CH_{2\ trans}$, J=17.33, 1.68, 1.68), 5.20 (1H, brd, C−H_A, J=5.68), 5.27 (1H, ddd, $=CH_{2\ cis}$, J=10.38, 1.65, 1.65), 6.32 (1H, ddd, C−H_X, J = 17.21, 10.04, 5.17), 6.53 (1H, s, C3−H), 6.67 (1H, s, C6−H), 6.80−7.15 (4H, m, B ring). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 41.68 (C−H_A), 57.56 ($-OCH_3$), 58.00 ($-OCH_3$), 58.42 ($-OCH_3$), 99.37 (C3), 114.64 (C6), 117.78 (C1), 118.19 ($=CH_2$), 122.00 (C3′), 122.74 (C1′) 129.17 (C5′) 129.80 (C4′), 130.71 (C6′), 140.51 (C−H_X), 145.14 (C5), 149.92 (C2), 151.61 (C4), 155.27 (C2′).

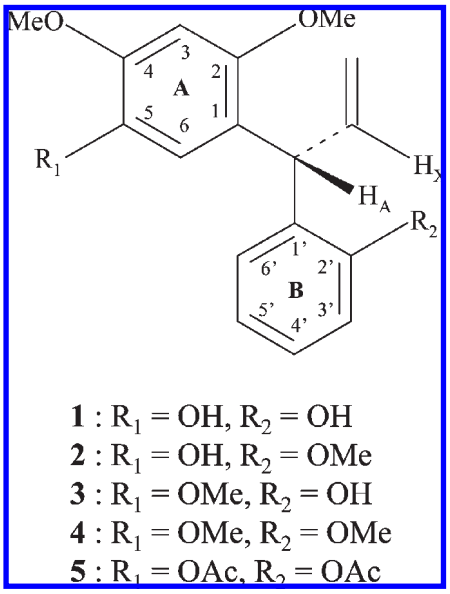

Figure 1. Chemical structures of compounds **1**−**5**: latifolin (**1**), 2′-*O*-methyllatifolin (**2**), 5-*O*-methylatifolin (**3**), latifolin dimethyl ether (**4**), and latifolin diacetate (**5**).

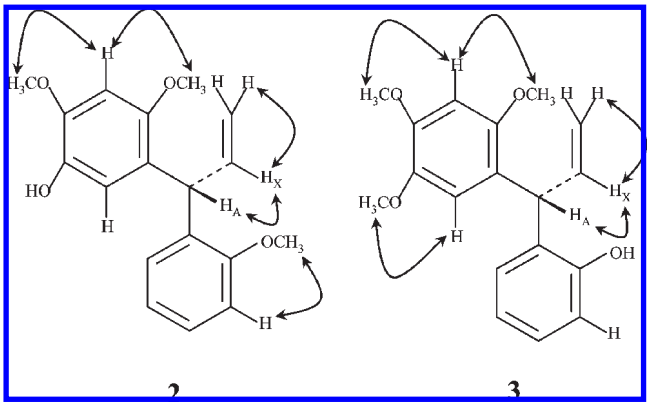

Figure 2. NOESY correlations of **2** and **3**. Numbers **2** and **3** refer to the compounds shown in **Figure 1**.

Latifolin Dimethyl Ether (4). MS, *m*/*z* (relative intensity): 314 $[M]^+$ (100), 299 (15), 283 (19), 267 (6), 253 (5), 241 (5), 225 (6), 207 (7), 191 (11), 181 (25), 175 (22), 165 (15), 151 (32), 145 (31), 131 (23), 121 (43), 115 (31), 107 (14), 105 (14), 91 (54), 77 (25), 69 (33), 55 (9).

Acetylation. A total of 50 mg of **1**, isolated in a previous study (*12*), was dissolved in pyridine (2.0 mL) and added to acetic anhydride (2.0 mL). The solution was stored overnight at room temperature and was then extracted by partition extraction with EtOAc.

Latifolin Diacetate (5). MS, *m*/*z* (relative intensity): 370 $[M]^+$ (15), 328 (100), 311 (1), 297 (5), 285 (47), 270 (11), 255 (81), 237 (4), 227 (6), 211 (6), 191 (5), 181 (9), 167 (21), 154 (34), 139 (10), 133 (12), 131 (22), 115 (11), 107 (11), 91 (9), 77 (11), 69 (21), 55 (7).

Antitermite Test. *Termite*. A colony of *R. speratus* Kolbe was collected from Tsuruoka, Japan, in May of 2008. The colony was maintained in a dark room at 27±1 °C and 70% relative humidity (RH) until initiation of the test.

Mortality Rate and Mass Loss. These tests were carried out according to previously described methods (*14*, *15*). Test samples (compound **1** and its derivatives) dissolved in acetone to make a 3% (w/w) solution were applied to paper discs (thickness, 1.5 mm; 8 mm diameter; Advantec, Tokyo, Japan). The paper discs were dried overnight in a vacuumed desiccator and placed on 2.0 g of sterile sand in a Petri dish (50 mm diameter). A total of 10 termites were placed into each Petri dish (six replicates), and the sand was moistened to supply water.

Table 1. Statistical Analysis of Bioactivity of Compound **1** and Its Derivatives[a]

			compounds				
			1	**2**	**3**	**4**	**5**
termite	mortality	percent mortality (mean ± SD)	26.7 ± 13.7 A	46.7 ± 13.7 B	30.0 ± 8.9 A	56.7 ± 18.6 B	53.3 ± 5.2 B
	mass loss	mass loss of paper disk (mg ± SD)	0.39 ± 0.13 A	0.82 ± 0.14 B	1.29 ± 0.14 C	1.27 ± 0.24 C	1.13 ± 0.08 C
fungus	*T. versicolor*	growth in diameter (mm ± SD)	15.0 ± 4.1 A	60.0 ± 1.3 BC	62.3 ± 2.5 C	60.8 ± 1.2 C	56.2 ± 5.4 B
	F. palustris		32.5 ± 5.3 A	42.5 ± 1.7 B	52.3 ± 0.96 C	57.0 ± 5.9 C	51.3 ± 2.5 C
	R. oryzae		ns	ns	ns	ns	ns
	C. cladosporioides		26.2 ± 2.6 A	30.0 ± 0.89 B	29.2 ± 1.2 B	29.3 ± 2.2 B	28.7 ± 0.52 B

[a] Numbers **1**−**5** refer to the compounds shown in **Figure 1**. The same letters are not significantly different. LSD, $p < 0.05$. ns = not significant.

The Petri dish with the test sample was placed in a dark room for 14 days. Survival of termites was counted each day to determine mortality rates. The mass loss (weight loss of the paper disk) was used to evaluate antifeedant activity of samples toward termite. The mass loss was determined by subtracting post-treatment from pretreatment paper disk weights, which had been vacuum-dried overnight. From the mortality rate and mass loss of compound **1**, the relative mortality and relative mass loss were expressed, respectively. In control experiments, termites were exposed to paper discs treated only with acetone.

Antifungal Test. *Fungus*. In this study, we used the following fungi, provided by the Natural Institute of Technology and Evaluation Biological Resource Center (NBRC) (Tokyo, Japan): *T. versicolor* (NBRC: 30340), *F. palustris* (NBRC:30339), *Rhizopus oryzae* (NBRC:31005), and *Cladosporium cladosporioides* (NBRC:6348). These fungi are routinely chosen for antifungal test in Japan Industrial Standard (JIS) K1571.

Growth Inhibition Rate of Fungi. The test was carried out according to the method previously reported (*16*). Test samples (compound **1** and its derivatives) were dissolved in acetone, and 5.0 μg/cm^2 was applied to the surface of potato glucose agar (PGA) media in Petri dish (90 mm diameter) and then air-dried. The edge of each precultured fungus colony was cut with a cork-borer (5 mm diameter), and the trimmed colony section was placed in the center of the media.

The fungi were cultured in the dark in an incubator at 25.5 °C. The cultivation period for the each species of fungi was 5 days (*T. versicolor*), 14 days (*F. palustris*), 36 h (*R. oryzae*), and 20 days (*C. cladosporioides*).

As for the control experiment, acetone (300 μL) alone was applied to PGA media and this treatments (six replicates) was carried out in parallel with the test sample treatments. The diameter of the mycerial growth was measured periodically. The growth and inhibition rates were calculated as the ratio between the relative growth to the control treatment sample.

Statistical Analysis. Test samples are compared using analysis of variance (ANOVA), and means were separated using a protected Fisher least-significant difference (LSD) test ($p < 0.05$; SPSS 10.0, SPSS, Inc.). The LSD means separations test followed transformation to arcsine square root percent mortality. The actual percent mortality is reported in the **Table 1**.

RESULTS

Antitermite Activity of Compound 1 and Its Derivatives. After 14 days, the mortality rate from compound **1** was 26.7%. Termite mortality rates of each sample during the test period (14 days) are expressed relative to the mortality for compound **1** (1.00) in **Figure 3**. The relative mortality rates at the final days of this test from exposure to compounds **2**, **3**, **4**, and **5** were 1.75, 1.13, 2.13, and 2.00, respectively. Therefore, among the derivatives, the insecticidal activity of compounds **2**, **4**, and **5** were 2-fold larger than compound **1**. The activity of compound **3**, however, was the same level as compound **1**. The mortality rate from dimethylated (**4**) and diacetylated (**5**) derivatives rapidly increased from the later stage compared to other samples in the test period.

The mass loss because of exposure to compound **1** and the control was 0.39 and 7.67 mg, respectively. The mass loss of each samples is also expressed relative to the mass loss for compound **1** (1.00) in **Figure 4**. The relative mass loss of compounds **2**, **3**, **4**, and **5** were 1.98, 3.25, 3.42, and 2.89, respectively. Compound **1** elicited the least mass loss, followed by compound **2**, 2′-methylated B ring, and other derivatives. The mass loss because of dimethylated (**4**), diacetylated (**5**) derivatives and compound **3** was approximately 3-fold greater than compound **1**.

Antifungal Activity of Compound 1 and Its Derivatives. The antifungal activity of compound **1** and its derivatives is shown in **Figure 5**. The inhibition rates of white-rot fungi (*T. versicolor*) for compounds **1**, **2**, **3**, **4**, and **5** were 79.1, 16.5, 13.2, 15.3, and 21.8%, respectively. The inhibition rates of brown-rot fungi (*P. palustris*) for compounds **1**, **2**, **3**, **4**, and **5** were 37.5, 18.3, −0.5, −9.6, and 1.4%, respectively. Therefore, derivatives appear to have reduced activity; in particular, the activity level of compound **2** decreased 50% in relation to compound **1**, and the others (compounds **3**, **4**, and **5**) had negligible inhibiting effect. The antifungal activity against *R. oryzae* and *C. cladosporioides* for compound **1** and its derivatives was relatively low compared to white- and brown-rot fungi.

Compound **1** showed the highest level of activity against all fungi, while the activity levels of all derivatives were significantly lower than that of compound **1**.

DISCUSSION

Relationship of Neoflavonoids and the Bioactivity. It has been reported that the termite mortality rate from isorhapontigenin (stilbene group) is increased when the compound is methylated (*17*). By comparing the activity of deacetylgedunin, 17-hydroxyazadiradione, nimbandiol, and their derivatives, it has been suggested that the hydroxyl group is responsible for the termite antifeedant activity (mass loss) (*18*). Ohmura et al. (*19*) observed that the level of termite (*Coptotermes formosanus* Shiraki) antifeedant activity of some flavonoids (quercetin, taxifolin, and naringetin) was influenced by the position of hydroxyl groups.

In this study, except for compound **3**, the mortality rate of compounds **2**, **4**, and **5** in the final days from exposure to the methylated and acetylated derivatives were twice as high as compared to that of compound **1**. There was no significant difference in the termite mortality level for derivatives with methylation at C-5 of the A ring. On the other hand, a high mortality rate was observed following methylation at C-2′ of the B ring. As previously described, the mortality of compounds **2**, **4**, and **5** increased 2-fold compared to compound **1** (**Figure 3**). However, a different pattern of mortality was observed here, in which the mortality rate of compound **2** increased steadily, while the mortality rate of compounds **4** and **5** increased sharply 7 days after initiation of the test (particularly for compound **5**). In other words, performance in insecticidal activity was retarded on dimethylated (**4**) and diacetylated (**5**) derivatives.

The termite mass loss caused by derivatives was 2-fold greater than compound **1**. In comparing the mass loss from compounds **2** and **3**, methylation at C-5 of the A ring induced greater mass loss than methylation at C-2′ of the B ring.

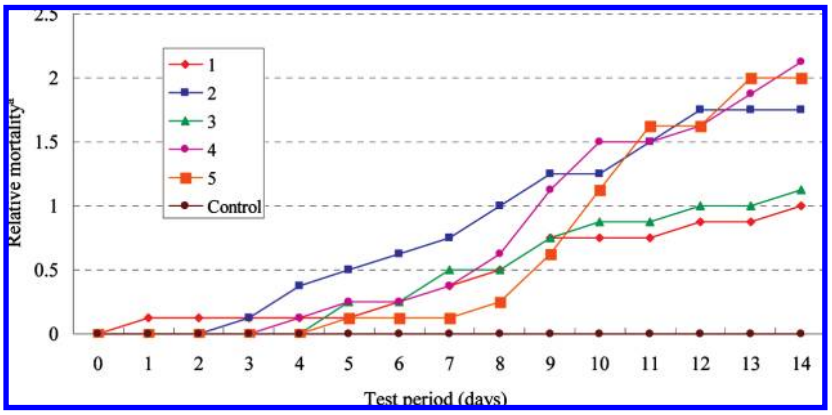

Figure 3. Termite mortality rate of compound **1** and its derivatives. Numbers **1**−**5** refer to the compounds shown in **Figure 1**. Mortality rate (%) = number of dead termites after 14 days of the tests/number of initial termites of the tests × 100, Relative mortality = mortality rate of each sample (%)/mortality rate of compound **1** (%) × 100.

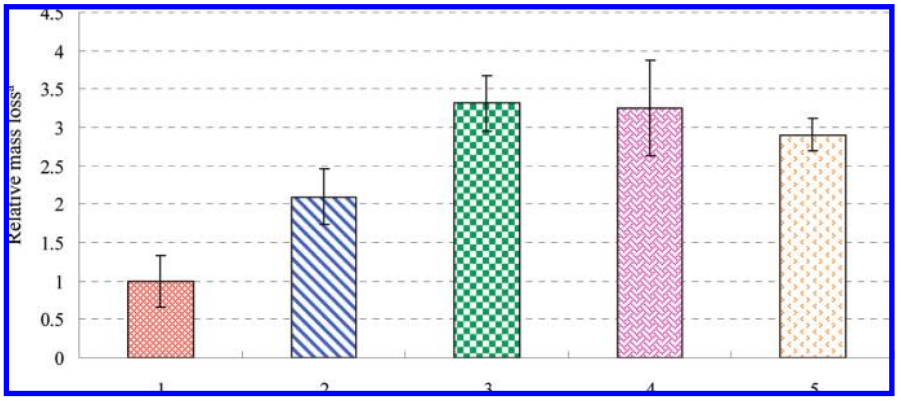

Figure 4. Mass loss of compound **1** and its derivatives. Numbers **1**−**5** refer to the compounds shown in **Figure 1**. Mass loss (%) = (pretreatment paper disk weight (mg) − post-treatment paper disk weight (mg))/pretreatment paper disk weight (mg)×100. Relative mass loss = mass loss of each sample (%)/mass loss of compound **1** (%) × 100. The error bar represents ±standard deviation (SD), which was calculated from the mass loss (%).

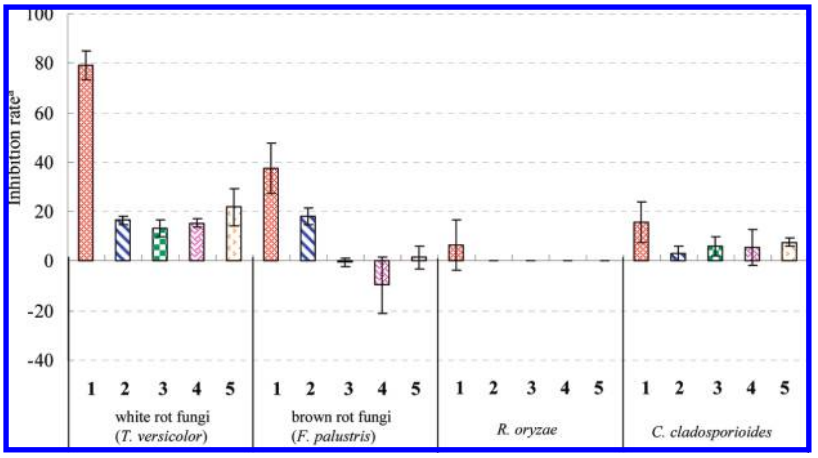

Figure 5. Fungal growth inhibition rate of compound **1** and its derivatives. Numbers **1**−**5** refer to the compounds shown in **Figure 1**. Growth rate (%) = mycelial growth in diameter of each sample (mm)/mycelial growth in diameter of the control (mm) × 100. Inhibition rate (%) = 100 − growth rate (%). The error bars represent ±SD.

In relating termite mortality and mass loss, it was found that, as the mortality rate of compound **2** became larger, then the mass loss was greater compared to compound **1**. Thus, for compound **2**, the increased mortality rate corresponded well with the increased mass loss. The mass loss from compound **3** was 3 times greater than compound **1**. However, the mortality rate from compound **3** did not increase considerably. That is, compound **3** had lower toxicity and antifeedant activity for termite than compound **2**. This finding might be due to methylation at C-5 of the A ring changing the antitermite activity of compound **1**. Therefore, it was suggested that the C-5 hydroxyl group of compound **1** contributed to the antifeedant and insecticidal activities more than the C-2′ hydroxyl group.

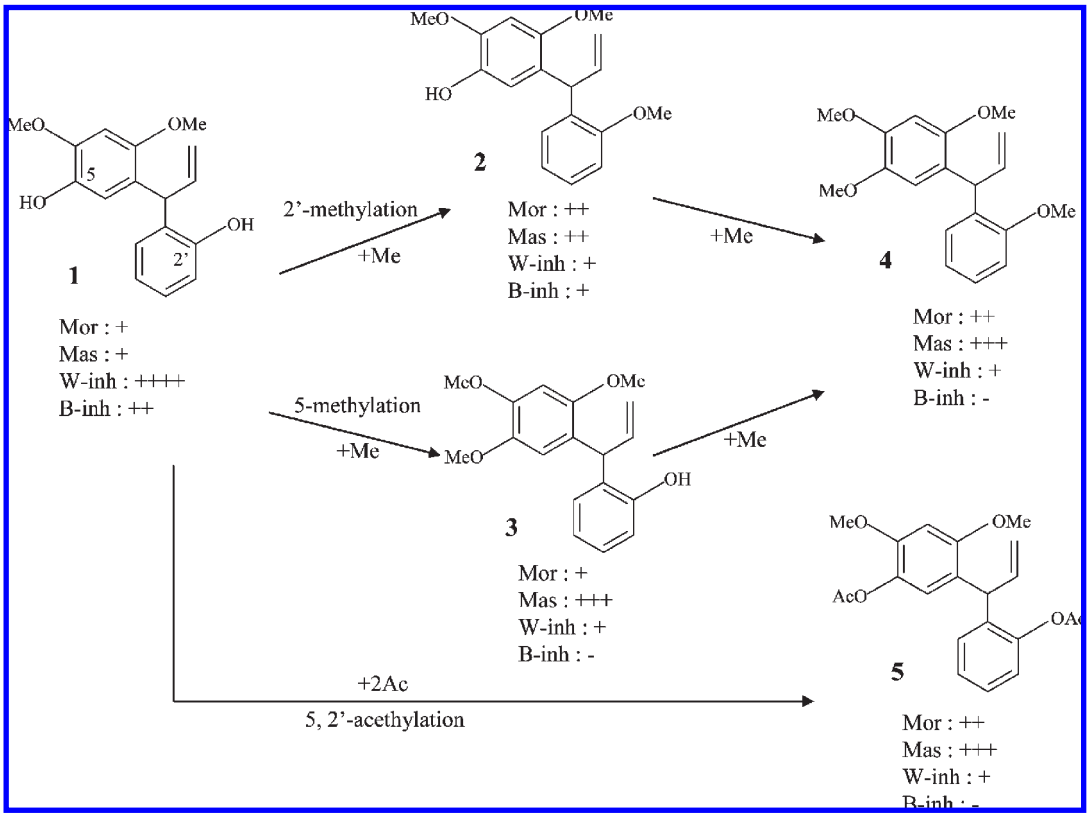

Figure 6. Bioactivity of compound **1** and its derivatives. Numbers **1**−**5** refer to the compounds shown in **Figure 1**. Mor means the relative mortality of termites in **Figure 3**: 0.5 < + < 1.5 and 1.5 < ++ < 2.5. Mas means the relative mass loss of termites in **Figure 4**: + < 1.5, 1.5 < ++ < 2.5, and 2.5 < +++. W-inh and B-inh mean the inhibition rates of white-rot and brown-rot fungi in **Figure 5**: 10.0 < + < 30.0 (%), 30.0 < ++ < 50.0 (%), 50.0 < +++ < 70.0 (%), and 70.0 (%) < ++++.

On the other hand, the mortality rate from compounds **4** and **5** increased sharply on the 7th day of the test and the level of activity became larger. Furthermore, the mass loss at the 14th day from compounds **4** and **5** was 3 times greater than for compound **1**. It is known that during termite metabolism, syntrophic acetate-oxidizing microbes in the termite body cause demethylation of vanillin and syringil acid under anaerobic conditions (*20*−*22*). We hypothesize that compounds **4** and **5** changed into compound **1** through demethylation in the termite body, such as vanillin and syringil acid, after they were fed. This change eventually led to the mortality of termites. This hypothesis is well-supported by the results of compound **5**. In addition, compounds **4** and **5** might be more accumulated in the termite body compared to compound **1**, because compounds **4** and **5** had lower antifeedant activity than compound **1**. Therefore, it was considered that the results in the case of compounds **4** and **5** were caused by demethylation or deacetilation of the derivatives accumulated in the termite body.

Studies of antifungal activity of stilbenes and its derivatives have shown that the activity against white-rot fungi (*Coriolus versicolor*) and brown-rot fungi (*Gloeophyllum trabeum* and *Poria placenta*) is related to the hydrophobicity (*23*, *24*). In this study, it appears that the bioactivity is affected by the structure of the compounds, particularly relative to the presence and position of hydroxyl and methoxy groups, as well as their hydrophobicity.

In assessing the antifungal activity of these compounds, we found that the inhibition rates for white-rot fungi from all derivatives were lower than the rate for compound **1**. There was no difference in the inhibition rate from the monomethylated derivatives (at C-5 and C-2′), indicating that the growth inhibition is mediated equally by hydroxyl groups at C-5 and C-2′. Moreover, the inhibition rate of brown-rot fungi was reduced in response to the 5-methylated A ring relative to the 2′-methylated B ring, indicating that the activity was affected by the hydroxyl group at C-5 of the A ring.

The summarized bioactivity of compound **1** and its derivatives is shown in **Figure 6**, and the statistical analysis of antitermite and antifungal activity is shown in **Table 1**. The difference in bioactivity among compound **1** and its derivatives was significant.

In conclusion, the bioactivity of compound **1**, which is a neoflavonoid, depends upon the position of phenolic hydroxyl groups. This position is a necessary and effective property in estimating the performance of compound **1** because the hydroxyl group at C-5 of the A ring has been shown to have antitermite activities (mortality and mass loss). In addition, both C-5 and C-2′ hydroxyl groups in the A and B rings have antifungal activity against white- and brown-rot fungi. Further work is needed for other functional groups of latifolin to thoroughly determine any correlation between the bioactivity and chemical structure.

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