J Wood Sci (2000) 46:149–153

ORIGINAL ARTICLE

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Antifeedant activity of flavonoids and related compounds against the subterranean termite *Coptotermes formosanus* Shiraki

Received: February 12, 1999 / Accepted: May 19, 1999

Abstract Antifeedant activity of some flavonoids and their related compounds against the subterranean termite *Coptotermes formosanus* Shiraki was examined with no-choice tests and two-choice tests. The activities of these compounds were evaluated in relation to their chemical structures. All flavonoids tested showed antifeedant activity, whereas catechinic acid, possessing no A-ring or pyran ring in the molecule, showed feeding-preference activity. For the structure–activity relations, it was found that compounds containing two hydroxyl groups at C-5 and C-7 in A-rings showed high antifeedant activity. Furthermore, the presence of a carbonyl group at C-4 in the pyran rings of the compounds was necessary for the occurrence of high activity. 3-Hydroxyflavones and 3-hydroxyflavanones with 3′,4′-dihydroxylated B-rings exhibited higher activity than those with 4′-hydroxylated B-rings.

Key words Flavonoids · Termite · Antifeedant activity · Structure-activity relations

Introduction

Serious environmental pollution has been caused by our excessive dependence on synthetic chemicals. Recently, the application of insecticides such as organochlorines tends to be restricted due to the impacts on our health and the environment. In the case of termite control, new safer treatment procedures in wood and soil have been investigated for practical use. For example, physical barriers using basaltic particles[1,2] and stainless steel mesh[3] have been developed as substitutes for soil treatment with chemicals. Also, many pest control agents, such as pyrethroids from *Chrysanthemum cinerariaefolium* Bocquilon, have been developed from phytochemical leads. They are generally nonpersistent and show mild modes of action.[4] Thus, plant extracts may be promising alternatives for pest control agents in the future.

Flavonoids are widely distributed in plants, and it is assumed that they are related to the resistance toward attacks by insects and fungi in several plant species. For instance, taxifolin and aromadendrin show antifungal activity against several wood-rotting fungi.[5] Taxifolin also inhibits the growth of *Heliothis zea* larvae[6] and exhibits antitermitic activity against the West Indian drywood termite, *Cryptotermes brevis* (Walker).[7]

Recently, it was found that steam-treated Japanese larch [*Larix leptolepis* (Sieb. et Zucc.) Gord.] heartwood was severely attacked by the subterranean termites *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe).[8,9] In our previous work,[10,11] taxifolin was found to be the main feeding deterrent of the non-steamed larch wood and to be decomposed during steam treatment. The degradation of taxifolin would be one of the important factors for the severe attack to the steamed larch wood by *C. formosanus*. Because flavonoids such as taxifolin were easily extracted with water from larch wood,[12] they may be supplied by an easy procedure with low cost. Therefore, it is suggested that flavonoids have potential as termite control agents.

In this study, we investigated the antifeedant effects of some flavonoids and their related compound against *C. formosanus* to obtain a better understanding of the antitermite activity of flavonoids. Both choice tests and no-choice tests were used to assess the potency of each compound as an antifeedant.

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Part of this paper was presented at the 49th annual meeting of the Japan Wood Research Society, Tokyo, April 1999

Materials and methods

Proton nuclear magnetic resonance (^{1}H-NMR) and carbon 13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on JEOL Lambda-400 and Alpha-500 spectrometers. Fast atom bombardment (FAB) mass spectra were obtained using a JEOL HX-110A mass spectrometer.

Preparation of test materials

Kaempferol and catechin were purchased from Wako Pure Chemical Industries (Japan) and fisetin, phloretin, and myricetin from Extrasynthese (France). Quercetin was isolated from Japanese larch heartwood according to the procedure described by Takehara and Sasaya.[13] Its NMR and FAB mass spectra coincided with those in the literature.

Eriodictyol, genistein, sakuranetin, and isosakuranetin were isolated from the wood of *Prunus* species by the method described by Hasegawa and Shirato.[14] These NMR spectral data were in agreement with those already published.[15–17]

Taxifolin, aromadendrin, and naringenin were isolated from Japanese larch wood as follows. One kilogram of Japanese larch wood meal passing a 1-mm screen mesh was extracted with 4 l of MeOH for 48 h at room temperature. The MeOH extracts were dried on a rotary evaporator and dissolved in 2-butanone. The 2-butanone solution was evaporated under reduced pressure, and the residue was sequentially extracted with *n*-hexane and ethyl acetate. The ethyl acetate-soluble fraction was separated by chromatography on a silica gel (70–230 mesh, Merck) column (450 × 32 mm i.d.) eluted with benzene/acetone (9:1, v/v). Taxifolin, aromadendrin, and naringenin were isolated by repeating recrystallization from a mixture of benzene and tetrahydrofuran. These compounds were identified by comparison of FAB mass spectra and melting points with authentic samples.[13]

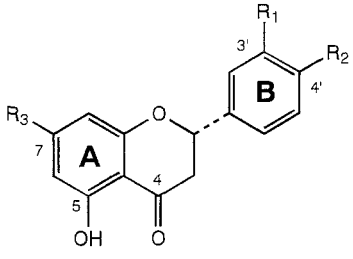
There are possibly several isomers as to the stereochemistry of flavonoids, but only one isomer has been identified so far as a natural product. The absolute configuration of the 2-position of flavanones is commonly 2*S*, and that of the enzymically derived 3-hydroxyflavanones is designated 2*R*.[18,19]

Catechinic acid was prepared by a base-catalyzed reaction of catechin at pH 12 and 100°C[20] and purified by Sephadex LH-20 column chromatography using ethanol as an eluent. The ^{1}H-NMR spectrum of the methyl ether derivative was in agreement with that published earlier.[20]

These compounds were submitted to bioassays. Their chemical structures are shown in Fig. 1.

Termite used for bioassays

The test termite, *C. formosanus*, was collected from a laboratory colony maintained in the Forestry and Forest Products Research Institute, Tsukuba, Japan. The colony has

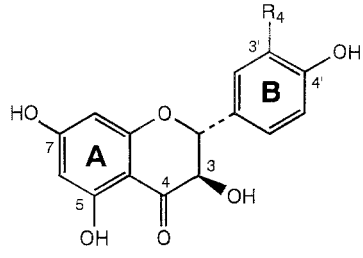


R1=OH, R2=OH, R3=OH : Eriodictyol

R1=H, R2=OH, R3=OH : Naringenin

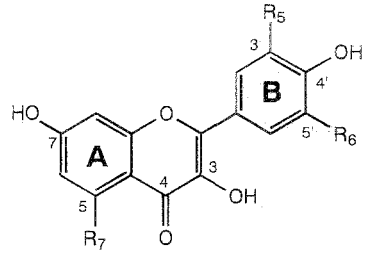
R1=H, R2=OH, R3=OMe : Sakuranetin

R1=H, R2=OMe, R3=OH : Isosakuranetin



R4=OH : Taxifolin

R4=H : Aromadendrin

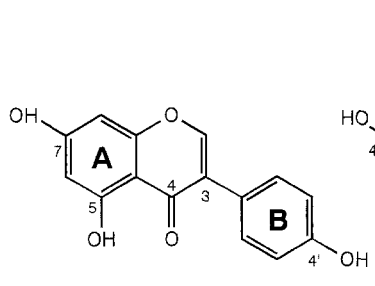


R5=H, R6=H, R7=OH : Kaempferol

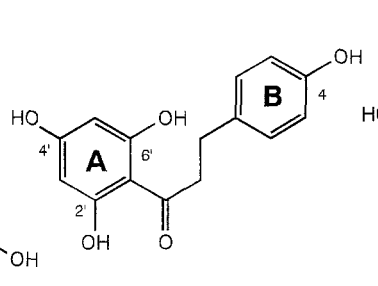
R5=OH, R6=H, R7=OH : Quercetin

R5=OH, R6=H, R7=H : Fisetin

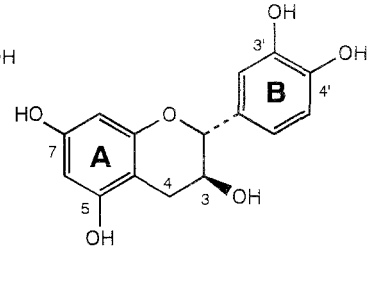
R5=OH, R6=OH, R7=OH : Myricetin



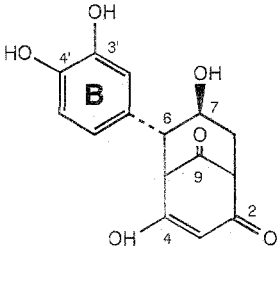
Genistein



Phloretin



Catechin



Catechinic acid

Fig. 1. Structures of test compounds

been reared on wood pieces in the dark at 28°± 2°C and 80% relative humidity (RH) for more than 15 years. Only pseudoergites (workers) above the third instar were used in the bioassays.

Antifeedant bioassay

No-choice and two-choice bioassays were conducted in this study. A test container was made of a plastic cup (rim diameter 6cm, bottom diameter 5cm, height 5cm) with a hard plaster bottom. The bottom was covered with 10g of sea sand (15–20 mesh; Junsei Chemical Co., Japan) and moistened with 2ml of deionized water. Paper discs (diameter 13mm; Whatman International) were permeated with 60μl of the MeOH solutions (1.0%, w/v) containing each of the test compounds. The treatment retention was 1.0% (w/w) per disc. The control discs were untreated. The discs were dried at 60°C for 12h followed by drying in a vacuum desiccator for 1 day. In the no-choice tests, a 15mm diameter plastic saucer holding a disc was placed on the center of the test container, and 50 termites were introduced into the container. In the two-choice tests, the two plastic saucers, which held a disc permeated with a sample solution and a control disc, respectively, were placed diagonally 12mm away from the center of the test container, and 100 termites were introduced. After 3 days the discs were taken out and dried in the same manner mentioned above to determine the weight loss of each disc. Three replications were carried out for each test compound for each of the two bioassays.

On the basis of the weight losses of the discs, the indices of the activity of the test compounds were calculated.[21] In the no-choice bioassay, the absolute coefficient of antifeedancy (A) was obtained by the following equation.

$$[(KK - EE)/(KK + EE)] \times 100\ (\%) \quad (1)$$

where KK and EE are the weight losses of the control and treated discs, respectively.

For the two-choice bioassay, the relative coefficient of antifeedancy (R) was calculated by the following equation.

$$[(K - E)/(K + E)] \times 100\ (\%) \quad (2)$$

where K and E represent the weight losses of the control and treated discs, respectively. The total coefficient of antifeedancy (T) is equal to A plus R. The maximum value of T reaches 200 for a complete antifeedant. All compounds tested were classified into the following five classes according to their T values; feeding preference ($T < 0$), class I ($0 \leq T < 50$), class II ($50 \leq T < 100$), class III ($100 \leq T < 150$), and class IV ($150 \leq T < 200$).

Results and discussion

Antifeedant activity of test compounds

As shown in Table 1, all the flavonoids tested showed antifeedant activities, whereas catechinic acid revealed feeding-preference activity. Four flavonoids obtained from Japanese larch wood (taxifolin, aromadendrin, quercetin, naringenin) showed high activity. Quercetin, taxifolin, and naringenin were classified into class IV, and aromadendrin was classified into class III. Other flavonoids were classified into class III, except for catechin, which was classified into class II.

Table 1. Antifeedant activity and functional groups of test compounds

Compounds	T value[c]	Class[d]	Hydroxylation			C-4 carbonyl group[e]	Other substituents
			A-ring	B-ring	Pyran ring		
Quercetin	162.6	IV	5, 7	3′,4′	3	○	
Taxifolin	154.2	IV	5, 7	3′,4′	3	○	
Naringenin	153.6	IV	5, 7	4′		○	
Isosakuranetin	144.1	III	5, 7			○	4′-Methoxyl (B-ring)
Aromadendrip	137.3	III	5, 7	4′	3	○	
Phloretin[a]	134.3	III					
Myricetin	132.7	III	5, 7	3′,4′,5′	3	○	
Sakuranetin	131.9	III	5	4′		○	7-Methoxyl (A-ring)
Eriodictyol	118.5	III	5, 7	3′,4′		○	
Genistein	117.5	III	5, 7	4′		○	
Fisetin	111.6	III	7	3′,4′	3	○	
Kaempferol	100.9	III	5, 7	4′	3	○	
Catechin	98.4	II	5, 7	3′,4′	3		
Catechinic acid[b]	−32.1	Feeding preference		3′,4′			

[a,b] The numbering systems are different from those of the other compounds (see Fig. 1)

[c] T is the total coefficient of antifeedancy which is equal to A plus R. A is the absolute coefficient of antifeedancy, $[(KK - EE)/(KK + EE)] \times 100(\%)$ of test compounds in the no-choice bioassay, where KK and EE represent the weight losses of the control and treated discs, respectively. R is the relative coefficient of antifeedancy, $[(K - E)/(K + E)] \times 100(\%)$ in the two-choice bioassay, where K and E represent the weight losses of the control and treated discs, respectively

[d] feeding preference ($T < 0$), class I ($0 \leq T < 50$), class II ($50 \leq T < 100$), class III ($100 \leq T < 150$), class IV ($150 \leq T < 200$)

[e] The mark (○) indicates the existence of C-4 carbonyl group in the molecule

Effects of A-ring structures on antifeedant activity

Methylation of hydroxyl groups at C-7 in the A-ring and at C-4′ in the B-ring of naringenin gives sakuranetin and isosakuranetin, respectively. They showed less antifeedant activity than naringenin, although the latter had higher activity than the former. These results suggest that hydroxyl groups at C-7 in A-rings have a larger effect on the activity than those at C-4′ in B-rings, and that C-7 hydroxyl groups of A-rings might be necessary for high antifeedant activity. Because quercetin showed higher antifeedant activity than fisetin, the hydroxyl group at C-5 in A-rings also plays an important role in the antifeedant activity.

Catechinic acid, which is a base-catalyzed reaction product from catechin, revealed feeding-preference activity. It has no A-ring or pyran ring in the molecule, although the B-ring remains unchanged (Fig. 1). This suggests the importance of the A-ring or pyran ring for the antifeedant activity.

Effects of B-ring structures on antifeedant activity

The growth of the corn earworm *Heliothis zea* was inhibited by *ortho*-hydroxylated flavonoids.[2] Thus, naringenin and kaempferol, which have a hydroxyl group only at C-4′ in B-rings, showed no antigrowth activity. In our study on termite antifeedancy, both naringenin and kaempferol showed antifeedant activity. Quercetin showed higher antifeedant activity than kaempferol, indicating that two vicinal phenolic hydroxyl groups on B-rings would be necessary for enhancing the activity. This tendency was also observed in the relation between taxifolin and aromadendrin. In contrast, eriodictyol showed lower activity than naringenin, although the former is comprised of a 3′,4′-dihydroxylated B-ring and the latter is comprised of a 4′-hydroxylated B-ring. Both eriodictyol and naringenin have no hydroxyl group at C-3 in pyran rings, whereas quercetin, kaempferol, taxifolin, and aromadendrin contain it in the molecules. Therefore, it is assumed that the presence of a hydroxyl group at C-3 in pyran rings enhances the effect of the hydroxyl groups at C-3′ in B-rings on the antifeedant activity.

Myricetin showed lower antifeedant activity than quercetin. This result shows that 3′,4′-dihydroxylated B-rings are more effective than 3′,4′,5′-trihydroxylated ones.

Effects of pyran ring structures on antifeedant activity

A dihydrochalcone, phloretin, showed less antifeedant activity than naringenin. This compound has the same structure as naringenin except for the absence of the pyran ring. From this result, the pyran ring was found to be important for antifeedant activity. Sandermann and Dietrichs[22] classified the antitermitic substances into three structural types (i.e., stilbenes, quinones, and pyran derivatives). The importance of the pyran ring of flavonoids to the activity confirmed in this study is in good agreement with their report.

Taxifolin with the carbonyl group at C-4 showed higher activity than catechin with the methylene group at C-4. Furthermore, only catechin was placed in class II, whereas all other flavonoids tested, having the carbonyl group at C-4, were in class III or IV. Therefore, the carbonyl group at C-4 in pyran rings is considered necessary for high antifeedant activity.

Conclusions

Choice and no-choice tests were conducted to evaluate the antifeedant activity of some flavonoids and their related compound against the subterranean termite *C. formosanus*. All flavonoids tested showed antifeedant activities. On the other hand, only catechinic acid, which possesses no A-ring or pyran ring in the molecule, showed feeding-preference activity. From the results, the following conclusions concerning structure-activity relations have been obtained.

1. Both C-5 and C-7 hydroxyl groups in A-rings are necessary for high activity.
2. The 3-hydroxyflavones and 3-hydroxyflavanones with 3′,4′-dihydroxylated B-rings show higher activity than those with 4′-hydroxylated B-rings.
3. The presence of the carbonyl group at C-4 in pyran rings is essential for the high activity.
4. Cleavage of the ether linkage of the pyran ring decreases the activity.

These facts suggest that some flavonoids such as quercetin and taxifolin might be useful for termite control agents, because they are abundant in plants.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research (B) (10556042, 1998-2000) from the Ministry of Education, Science and Culture of Japan. The authors thank Mr. K. Hashida and members of the Wood Extractives Laboratory, Forestry and Forest Products Research Institute, for providing the catechinic acid and some of the flavonoids used in this study.

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