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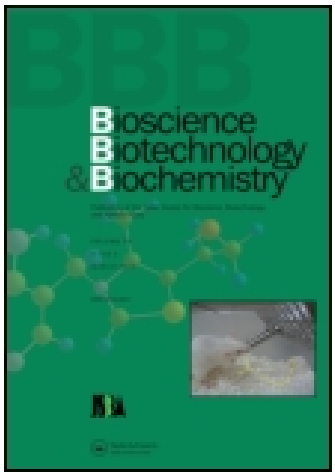

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Syntheses and Biological Activities of Dihydro-5,6-dehydrokawain Derivatives

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Syntheses and Biological Activities of Dihydro-5,6-dehydrokawain Derivatives

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The syntheses and biological activities of dihydro-5,6-dehydrokawain derivatives against plant pathogenic fungi and termites were investigated. Dihydro-5,6-dehydrokawain was isolated by a simple method without chromatography from the leaves of *Alpinia speciosa* K. SCHUM. The white crystalline compound obtained was identified as dihydro-5,6-dehydrokawain (1) by instrumental analyses. 4-Hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one (3) was prepared by hydrolyzing dihydro-5,6-dehydrokawain. Three dihydro-5,6-dehydrokawain derivatives were synthesized by reacting 3 with phosphoric agents.

Among the synthesized compounds, dimethyl [6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl]phosphorothionate (4) had the strongest antifungal activity of 91% at 100 ppm against *Corticium rolfsii*.

Key words: dihydro-5,6-dehydrokawain; dimethyl [6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl]phosphorothionate; 4-hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one; antifungal activity; *Corticium rolfsii*; *Alpinia speciosa* K. SCHUM

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Alpinia speciosa K. SCHUM is one of the Zingibraceae species that contains various phenolic compounds. Cardamomin and alpinetin have been obtained from its seeds.[1] Flavokawain B, dihydroflavokawain B, dihydro-5,6-dehydrokawain (**1**), and 5,6-dehydrokawain (**2**) have been isolated from rhizomes,[2,3] compounds **1** and **2** being found to have an antiulcer effect and antiplatelet action.[4,5] The insecticidal and antifungal activities of dihydro-5,6-dehydrokawain derivatives have not previously been studied. It is cost-prohibitive to isolate **1** by chromatography, so we investigated the simple isolation of compound **1** from leaves soaked in boiling water. We prepared phosphorus compounds because most of them have insecticidal properties. Dihydro-5,6-dehydrokawain phosphorus derivatives were examined for their insecticidal and antifungal activities against *Coptotermes formosanus* and *Pythium* sp., *Corticium rolfsii*. This paper presents the correlation between the chemical structure and biological activities of dihydro-5,6-dehydrokawain derivatives.

Materials and Methods

Materials. Fresh leaves of *A. speciosa* were collected at Nishihara in Okinawa. 5,6-Dehydrokawain was isolated from rhizomes of *A. speciosa* by using a method similar to that reported by Itokawa *et al.*[2] Dimethyl chlorothiophosphate and the other reagents used were of the highest grade commercially available.

Thin layer-chromatography. Each synthesized product was purified by using benzene–chloroform (1 : 1, v/v) on a pre-coated silica gel glass plate (Kieselgel 60 PF_{254}, 20 × 20 cm, 2.0 mm layer thickness, Merck). The objective compounds were detected under ultraviolet light and were recovered from benzene.

Instrumental analyses. The structure of each compound was confirmed by IR (Analect RFX-30), and by ^{1}H-NMR and ^{13}C-NMR in $CDCl_3$ with TMS as an internal standard (JEOL JNM-GSX 270). The ^{1}H-NMR and ^{13}C-NMR spectra were measured at 270 and 68 MHz, respectively. EI mass spectra were recorded with a JEOL JMS-DX 300 spectrometer, using 70 eV as the ionizing energy. HPLC analysis was performed by using a JASCO HPLC system equipped with a UV detector. Separation was carried out by using a reversed-phase Megapak sil C-18 column (JASCO) (10 × 250 mm) with a 10-μm particle size. The mobile phase was H_2O–MeOH (30 : 70) at a flow rate of 3.0 ml/min, the eluate being detected at 280 nm.

Insecticidal test. Termites (*Coptotermes formosanus*) were used as the test insects. An acetone solution containing 0.1 mg or 1.0 mg of a test compound was permeated on to filter paper (8.5 cm i.d.).

The permeated solution was exposed to the air for 24 h and almost completely volatilized. The test paper was placed on a petri dish (9 cm i.d.) and thirty termites were placed in the dish. These termites were observed for 14 days, and the number of dead insects was counted. The test paper was kept moist by water during the period of testing. The bioassay was conducted on three replicates, the data obtained being combined to calculate the mean value. Insecticide mortality rate was calculated as a percentage in comparison with the control group of termites.

Antifungal test. Pythium sp. and *Corticium rolfsii* were used as sources of pathogenic fungi. Each was cultured in potato dextrose agar (1.5% agar) for 5 days at 27°C and then tested. The antifungal activity was measured by the agar dilution method reported in the previous paper.[6]

Contents of dihydro-5,6-dehydrokawain and 5,6-dehydrokawain in various parts of Alpinia speciosa. Fresh leaves, stems, and rhizomes of *A. speciosa* were cut into pieces and soaked in ethanol for 1 week, before the crude extracts were analyzed by HPLC. Each peak area was measured at 12.9 min for compound **1** and at 17.7 min for **2**. The amounts of compounds **1** and **2** were calculated as percentages in comparison with the fresh weight of the original material.

Simple isolation method for dihydro-5,6-dehydrokawain (**1**). Fresh leaves of *A. speciosa* (2.2 kg) were cut into pieces and soaked in boiling water for 20 min. The crude extract was filtered, and the resulting filtrate was mixed with an equivalent amount of chloroform. The chloroform layer was evaporated *in vacuo* and yielded yellowish solid matter (2.95 g). This matter was dissolved in boiling water (200 ml) to filter out the insoluble material. The resulting filtrate was crystallized at 8°C for 18 h, and the white crystals formed were dried in a desiccator to yield 0.9 g. Melting point (mp), IR, and NMR spectral data for the crystals were in agreement with the data for dihydro-5,6-dehydrokawain (**1**).[2,3]

Hydrolysis of dihydro-5,6-dehydrokawain (**1**). Dihydro-5,6-dehydrokawain (**1**; 0.48 mg, 2.1 mmol) was dissolved in conc. HCl (40 ml) and stirred for 18 h at room temperature. After the completion of the reaction, distilled water (200 ml) was added to the reaction mixture, which was then crystallized at 8°C for 18 h. The crystals formed were percolated in a glass filter and washed three times with a saturated NaCl solution (50 ml × 3). The resulting white crystals were dried in a desiccator to yield 0.39 g (87.2%) of 4-hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one (**3**), mp 141.9–

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143.5°C. IR ν_{max} (nujol) cm^{-1}: 3446 (O–H). ^{1}H-NMR δ ($CDCl_3$): 2.77 (2H, t, J=7.6 Hz, H-7), 2.95 (2H, t, J=7.6 Hz, H-8), 5.60 (1H, s, H-3), 5.95 (1H, s, H-5), 7.14–7.30 (5H, m, aromatic). ^{13}C-NMR δ ($CDCl_3$): 32.8 (s, C-8), 35.4 (s, C-7), 90.0 (s, C-3), 101.9 (s, C-5), 126.5 (s, C-12), 128.2 (s, C-10 and C-14), 128.6 (s, C-11 and C-13), 139.6 (s, C-9), 165.9 (s, C-6), 168.2 (s, C-4), 172.4 (s, C-2).

Syntheses of the dihydro-5,6-dehydrokawain derivatives. A mixture of 4-hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one (**3**; 165 mg, 0.76 mmol) and triethylamine (309 mg, 3.06 mmol) in dichloromethane (25 ml) was added dropwise to a stirred solution of dimethyl chlorothiophosphate (123 mg, 0.76 mmol) in dichloromethane (25 ml) at 10°C. The reaction mixture was stirred at 40°C for 3 h. After the completion of the reaction, the solution was evaporated *in vacuo*. The resulting residue was mixed with benzene (100 ml), and the precipitate was removed by filtration. The filtrate was evaporated *in vacuo*, and the residue formed was purified by the preparative TLC method just described to yield 31 mg (12.0%) of dimethyl [6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl]phosphorothionate (**4**). $n_D^{24.2}$ 1.5133. TLC R_f 0.42 by benzene–chloroform (1 : 1, v/v). IR ν_{max} (neat) cm^{-1}: 1736 (C=O), 1034 (P–O–C), 841 (P–O), 700 (P=S). EI mass spectrum (relative intensity, %) *m/z*: 340 (M^+, 23), 221 (10), 149 (10), 125 (15), 91 (100), 32 (33). ^{1}H-NMR δ ($CDCl_3$): 2.80 (2H, t, J=7.6 Hz, H-7), 2.99 (2H, t, J=7.6 Hz, H-8), 3.84 (6H, d, $^3J_{HCOP}$=14.0 Hz, 2×OC$\underline{H}_3$), 5.92 (1H, s, H-3), 5.98 (1H, s, H-5), 7.16–7.33 (5H, m, aromatic). ^{13}C-NMR δ ($CDCl_3$): 32.8 (s, C-8), 35.7 (s, C-7), 55.6 (d, $^2J_{COP}$=5.2 Hz, 2×O$\underline{C}H_3$), 99.5 (d, $^3J_{CCOP}$=6.2 Hz, C-3), 101.0 (d, $^3J_{CCOP}$=6.2 Hz, C-5), 126.6 (s, C-12), 128.3 (s, C-10 and C-14), 128.7 (s, C-11 and C-13), 139.5 (s, C-9), 162.8 (d, $^2J_{COP}$=7.3 Hz, C-4), 163.6 (s, C-6), 166.1 (s, C-2).

The same procedure was used to prepare compounds **5** and **6**.

Diethyl [6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl]phosphorothionate (**5**): Yield, 207 mg (73.7%). $n_D^{24.7}$ 1.5437. TLC R_f 0.37 by benzene–chloroform (1 : 1, v/v). IR ν_{max} (neat) cm^{-1}: 1736 (C=O), 1020 (P–O–C), 829 (P–O), 700 (P=S). EI mass spectrum (relative intensity, %) *m/z*: 368 (M^+, 30), 249 (14), 149 (10), 91 (100), 18 (25). ^{1}H-NMR δ ($CDCl_3$): 1.36 (6H, t, J=7.2 Hz, 2×$OCH_2C\underline{H}_3$), 2.79 (2H, t, J=7.8 Hz, H-7), 2.98 (2H, t, J=7.6 Hz, H-8), 4.22 (4H, m, 2×OC$\underline{H}_2CH_3$), 5.93 (1H, s, H-3), 6.00 (1H, s, H-5), 7.15–7.35 (5H, m, aromatic). ^{13}C-NMR δ ($CDCl_3$): 15.8 (d, $^3J_{CCOP}$=7.3 Hz, 2×$OCH_2\underline{C}H_3$), 32.8 (s, C-8), 35.6 (s, C-7), 65.8 (d, $^2J_{COP}$=6.2 Hz, 2×O$\underline{C}H_2CH_3$), 99.3 (d, $^3J_{CCOP}$=6.3 Hz, C-3), 101.1 (d, $^3J_{CCOP}$=7.3 Hz, C-5), 126.5 (s, C-12), 128.3 (s, C-10 and C-14), 128.6 (s, C-11 and C-13), 139.5 (s, C-9), 163.0 (d, $^2J_{COP}$=7.3 Hz, C-4), 163.6 (s, C-6), 165.9 (s, C-2).

[6-(2-Phenylethyl)-2-oxo-2*H*-pyran-4-yl]diphenylphosphinothionate (**6**): Yield, 89 mg (44.9%). $n_D^{24.9}$ 1.6076. TLC R_f 0.35 by benzene–chloroform (1 : 1, v/v). IR ν_{max} (neat) cm^{-1}: 1732 (C=O), 995 (P–O–C), 827 (P–O), 692 (P=S). EI mass spectrum (relative intensity, %) *m/z*: 432 (M^+, 30), 217 (100), 139 (50), 91 (50), 57 (31), 32 (40). ^{1}H-NMR δ ($CDCl_3$): 2.72 (2H, t, J=7.8 Hz, H-7), 2.92 (2H, t, J=7.8 Hz, H-8), 5.87 (1H, s, H-3), 5.98 (1H, s, H-5), 7.12–7.93 (15H, m, aromatic). ^{13}C-NMR δ ($CDCl_3$): 32.7 (s, C-8), 35.6 (s, C-7), 99.9 (d, $^3J_{CCOP}$=7.3 Hz, C-3), 101.7 (d, $^3J_{CCOP}$=6.3 Hz, C-5), 126.5 (s, C-12), 128.2 (s, C-10 and C-14), 128.6 (s, C-11 and C-13), 128.8 (d, $^2J_{CCP}$=11.4 Hz, 2×(C-2′ and C-6′)), 131.2 (d, $^3J_{CCCP}$=13.5 Hz, 2×(C-3′ and C-5′)), 132.74 (d, $^1J_{CP}$=111.1 Hz, 2×(C-1′)), 132.77 (d, $^4J_{CCCCP}$=3.1 Hz, 2×(C-4′)), 139.6 (s, C-9), 163.3 (d, $^2J_{COP}$=8.3 Hz, C-4), 163.4 (s, C-6), 165.6 (s, C-2).

Results and Discussion

Contents of dihydro-5,6-dehydrokawain (**1**) *and 5,6-dehydrokawain* (**2**) *found in various parts of Alpinia speciosa*

We checked the various parts of *A. speciosa* to determine which had the highest concentration level of **1**. Leaves, stems, and rhizomes of *A. speciosa* were soaked in ethanol, and each extracted solution was examined to elucidate the contents of **1** and **2** as a percentage by fresh weight of the original material. As shown in Table I, **1** was found mostly in the leaves (0.41%), while compound **2** was found in rhizomes (0.1%) with a scant amount of it in the leaves.

Simple isolation method for dihydro-5,6-dehydrokawain (**1**)

Leaves of *A. speciosa* were soaked in boiling water, and samples were taken every 5 min to be analyzed quickly by HPLC. The concentration of dihydro-5,6-dehydrokawain quickly increased in the first 5 min and more slowly after that. After about 20 min, the highest concentration was noted, and the soaking process was then discontinued. The crude boiling-water extract contained a substantial amount of dihydro-5,6-dehydrokawain (**1**) which was extracted by chloroform. The chloroform layer was almost pure dihydro-5,6-dehydrokawain (**1**), but contained a trace amount of impurities. A yellowish solid material was obtained by evaporating the chloroform layer, this being dissolved in boiling water again and the insoluble matter rapidly filtered out. The resulting filtrate was crystallized at 8°C to yield 0.9 g (0.04%) of dihydro-5,6-dehydrokawain (**1**). This isolation method for compound **1** is superior to others because it is not necessary to use column chromatography.[2)]

Table I. Contents of Dihydro-5,6-dehydrokawain (**1**) and 5,6-Dehydrokawain (**2**) in Various Parts of *Alpinia speciosa*

Compound	% of fresh weight		
	Leaves	Stems	Rhizomes
Dihydro-5,6-dehydrokawain (**1**)	0.41	0.08	0.35
5,6-Dehydrokawain (**2**)	0.01	0.02	0.10

Syntheses of the dihydro-5,6-dehydrokawain derivatives

The compounds were synthesized as shown in Fig. 1. Dihydro-5,6-dehydrokawain (**1**) was hydrolyzed by conc. HCl to yield 4-hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one (**3**) which has a hydroxy group instead of a methoxy group at position 4 of compound **1**.[7)] Compound **3** was reacted with dimethyl chlorothiophosphate, diethyl chlorothiophosphate, and diphenylphosphinothioyl chloride by using triethylamine as a base to yield three derivatives. The IR spectra of the compounds showed the existence of P=S, P–O, and P–O–C groups. ^{1}H-NMR and ^{13}C-NMR data showed similar chemical shifts in the values for the 6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl group.

Insecticidal activity

The insecticidal activity against termites (*Coptotermes formosanus*) is shown in Fig. 2. Dihydro-5,6-dehydrokawain (**1**) and its demethylated compound, 4-hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one (**3**), showed poor insecticidal activity at 1.0 mg and 0.1 mg/filter paper. Dimethyl [6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl]phosphorothionate (**4**) showed the strongest activity of 93%, next was **5** with 50% at 1.0 mg/filter paper, and **6** showed the least activity. The results suggest that small molecular substituents imparted stronger activity than large ones.

Antifungal activity of dihydro-5,6-dehydrokawain (**1**) *and its derivatives*

The antifungal activity of the dihydro-5,6-dehydrokawain derivatives against pathogenic fungi, *Pythium* sp. and *Corticium rolfsii*, is listed in Table II. Dihydro-5,6-dehydrokawain (**1**) showed stronger activity than 5,6-dehydrokawain (**2**). Compound **1** showed the strongest activity (71.6% at 100 ppm), next being dimethyl [6-(2-phenylethyl)-2-oxo-2*H*-pyran-4-yl]phosphorothionate (**4**) (49.7%) against *Pythium* sp. Dimethyl derivative **4** showed the

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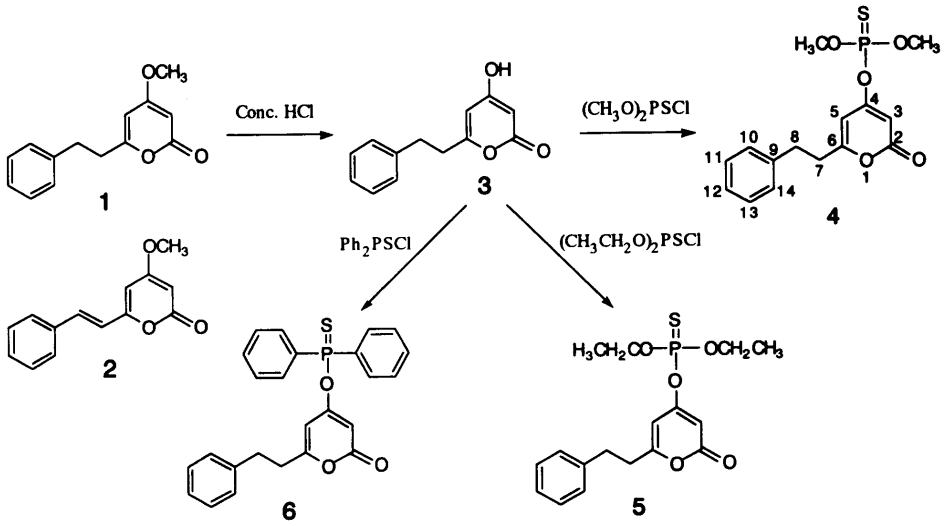

Fig. 1. Structures and Synthetic Routes for the Dihydro-5,6-dehydrokawain Derivatives.

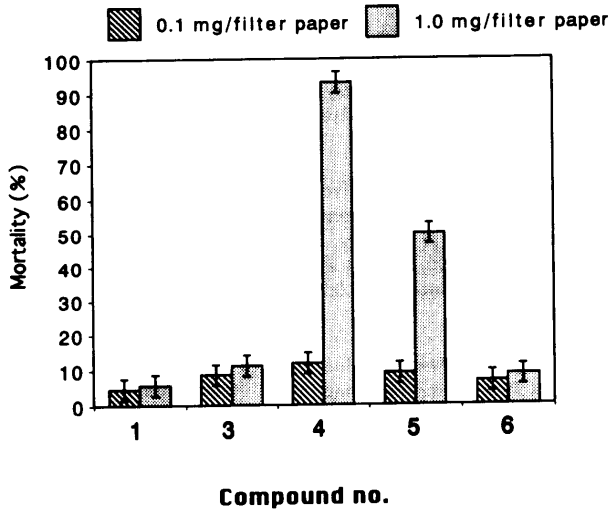

Fig. 2. Insecticidal Activity of the Dihydro-5,6-dehydrokawain Derivatives against *Coptotermes formosanus*.

1, dihydro-5,6-dehydrokawain; **3**, 4-hydroxy-6-(2-phenylethyl)-2*H*-pyran-2-one. Phosphorus esters: **4**, dimethyl; **5**, diethyl; **6**, diphenyl.

strongest activity (91.0%), next being dihydro-5,6-dehydrokawain (**1**) (64.2%) against *C. rolfsii*. Diphenyl derivative **6** showed the least activity. The activity of these derivatives in order of the most active compounds was dimethyl > diethyl > diphenyl, suggesting that small molecular substituents imparted stronger activity than large ones.

Dihydro-5,6-dehydrokawain (**1**) had strong antifungal activity and has been reported to have no toxic effect on mammals.[8] This indicates its possible use as a food antiseptic. Further studies are needed to investigate the antiseptic effect on various foods.

Table II. Antifungal Activity of Dihydro-5,6-dehydrokawain (**1**) and Its Derivatives

Compound[a]	Growth inhibition (%)			
	Pythium sp.		*Corticium rolfsii*	
	100 (ppm)	1000 (ppm)	100 (ppm)	1000 (ppm)
1	72	94	64	96
2	42	86	0	54
3	22	100	44	100
4	50	88	91	98
5	18	44	57	66
6	0	3	17	29

[a] Refer to Fig. 1 for the structures.

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