Termiticidal Activity of Diterpenes from the Roots of *Euphorbia kansui*

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Five ingenane compounds, **1**–**5**, kansuinins A and B, isolated from *Euphorbia kansui*, and their derivatives **7** and **9** were tested for termiticidal activity against the Japanese termite, *Reticulitermes speratus*. At 72 hours after treatment, the ingenane compounds **1** to **5** caused 100% mortality in *R. speratus* at 50, 25 and 12.5 μg/disk, respectively, except for compound **1**, which gave a mortality rate of (93.06 ± 5.56)% at 12.5 μg/disk. At 36, 48 and 60 hours after treatment, compounds **1** to **5** showed more termiticidal activity than kansuinins A and B and their derivatives. The kansuinins showed no or only slight activity against termites in the filter paper bioassay under the conditions tested compared with a solvent control.

Key words: Euphorbia kansui, Diterpenoid, Termiticidal Activity, *Reticulitermes speratus*

Introduction

The termite *Reticulitermes speratus* Kolbe, which is distributed throughout Japan, China and Korea, is of economic importance as a pest in Japan, and causes considerable damage to wooden structures and cellulosic materials (Cullinery and Grace, 2000). Current approaches to termite control mainly rely upon synthetic pesticides and traditional wood preservatives, for instance, chromium and arsenic salts, coal tar creosote, and pentachlorophenol (Serit *et al.*, 1992; Lee and Ryu, 2003). In view of their broad-spectrum toxicants, the environmental hazards associated with indiscriminate use of these compounds are likely to limit their future exploitation. This has led researchers to search for plant derivatives or botanical insecticides, that occur naturally in plants, usable for pest management (Isman, 2006). Some natural termiticidal products have been isolated from plants for the control of the Japanese termite (*R. speratus*), including four limonoids (Serit *et al.*, 1992), plant essential oils (Park and Shin, 2005), pterocarpans and pterocarpol (Morimoto *et al.*, 2006), whereas the data on botanical pesticides for termite control are very scant. During our course of screening antinematodal substances from *Euphorbia kansui*, we also found that the plant has termiticidal activity against the Japanese termite.

Euphorbia kansui is a herbal plant with a long history in medicine, which has been utilized as a remedy for the treatment of edema, ascites (Pharmacopoeia Commission, 1995) and cancers (Xu *et al.*, 1997; Xia and Li, 1999). It is distributed in northwestern and northern China, and its roots contain many biologically active substances that inhibit enzyme activity and the proliferation of embryonic cells (Wang *et al.*, 2002; Miyata *et al.*, 2004, 2006), and exhibit antitumour (Wu *et al.*, 1991) and antiviral activity (Zheng *et al.*, 1998). Although many biologically active compounds have been obtained from the plant, to our knowledge no other studies have reported the use of its compounds for pest control apart from our antinematodal research (Shi *et al.*, 2007, 2008).

Natural product-based termiticides may supply potent leads for the development of new commercial insecticides in the future. The termiticidal activities of diterpens obtained from *E. kansui* against the Japanese termite, *R. speratus*, were studied as part of a program aimed at searching for potential botanical termiticides as alternatives to traditional synthetic termiticides.

Materials and Methods

General experimental procedures

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were conducted with a Varian Unity Inova

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AS 600 instrument (Palo Alto, CA, USA) at 600 MHz and 150 MHz, respectively. Electrospray ionization mass spectroscopy (ESIMS) was performed by an API (atmospheric pressure ionization) III triple quadrupole mass spectrometer equipped with an ion spray interface (PE-Sciex; Thorn Hill, ON, Canada). Optical rotation was carried out on a Jasco DIP-360 polarimeter (Japan Spectroscopic Co., LTD., Tokyo, Japan). Infrared (IR) (KBr) spectra were obtained on a Jasco FT/IR-5000 spectrometer (Japan Spectroscopic Co.), and ultraviolet absorption (UV) spectra were measured by a Shimadzu MultiSpec-1500 spectrophotometer (Shimadzu Corporation, Tokyo, Japan). High performance liquid chromatography (HPLC) was performed on a Hitachi L-6250 Intelligent Pump (Hitachi High-Technologies Corporation, Japan) with an inertsil ODS-3 (Octadecylsilica) column (5 μm, 4.6 × 250 mm, GL Sciences Inc., Tokyo, Japan) and an L-7420 UV&Vis detector (Hitachi High-Technologies Corporation). Column chromatography was performed on silica gel 60 (Merck, 0.040–0.063 mm) and on reverse-phase silica gel (Millipore Preparative C18, 125 Å, 55–105 μm). Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel 60 F_{254} plates (Merck, 0.25 mm and 1.00 mm, respectively), and reverse-phase TLC was performed on RP-18 F_{254s} (Merck).

Plant material and termites

The dried roots of *Euphorbia kansui* Liou were purchased from the traditional Chinese Medicine Supermarket in Xi'an, Shaanxi Province of China. A type specimen was deposited at the College of Life Science of Northwest University in Xi'an, China.

Reticulitermes speratus (Kolbe) was collected from Okayama University experimental forest field. Termite-infested pine logs were maintained in plastic containers (350 × 250 × 150 mm) at 85% relative humidity until testing.

Termite bioassay

Termiticidal activity was evaluated as follows. Each solution was pipetted onto filter paper disks (Advantec Toyo No. 2, 16 mm Ø), at sample masses of 50, 25 and 12.5 μg in 20 μl acetone. After the solvent had evaporated at room temperature, treated and untreated filter papers (controls comprised only 20 μl evaporated acetone) were placed into vials (45 mm high and 18 mm in diameter). After the addition of 20 μl of distilled water, 16 worker termites at the third instars (based on termite size) were placed in each vial and kept at (26 ± 1) °C at 85% relative humidity. It had been previously determined that treatment of filter paper with 20 μl acetone as a control had no discernable effect on termite mortality. From 36 h after treatment, the number of dead termites was recorded every 12 h, and the cumulative mortality (%) was calculated as the number of dead termites/total number of termites ×100%.

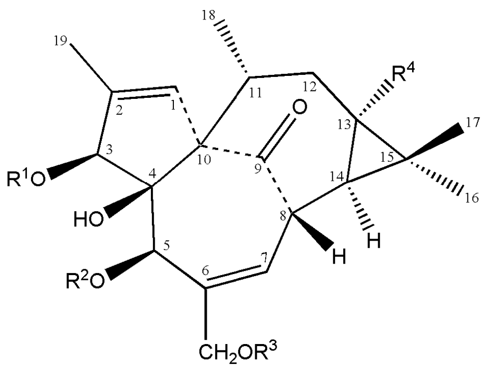
Analysis of variance (ANOVA) (JMP. IN 5.1, 2004) was conducted on these results and was followed by Tukey's honest significant difference (HSD) test ($P < 0.05$).

Extract and separation

Dried roots (10 kg) of *E. kansui* were ground and macerated with 20 l EtOH for two weeks. After concentration, the residue (239.68 g) was suspended in water, followed by separation with hexane and EtOAc. The hexane fraction (113.82 g) was separated by silica gel column chromatography eluted with a graded series of *n*-hexane and EtOAc, and fraction VI (4.087 g) was again subjected to silica gel column chromatography eluted with $CHCl_3$/MeOH (97:3, v/v). 165 mg were obtained and purified by reverse-phase column chromatography eluted with MeOH and H_2O (65:35, v/v) to yield compound **6** (60 mg).

The EtOAc fraction (88.04 g) obtained was applied to a silica gel column with a series of gradients of *n*-hexane/EtOAc and EtOAc/MeOH to give five fractions, fraction II (6.51 g) of which was again subjected to a silica gel column eluted with $CHCl_3$/MeOH (99:1, v/v), monitored by TLC, to give eight subfractions. Subfraction V (1.488 g) was repeatedly subjected to reverse-phase column chromatography, eluted with a graded series of MeOH and H_2O, monitored by ODS TLC and purified further by HPLC, when detection was difficult, to yield compound **8** (175 mg). Compounds **6** (38 mg) and **8** (40 mg), dissolved in 1 ml $CHCl_3$, were allowed to stand at room temperature [in (30 ± 1) °C] for two weeks; then they were subjected to preparative TLC, developed with $CHCl_3$/MeOH (99:1, v/v), to give rise to compound **7** (8 mg) and compound **9** (15 mg).

Five ingenol-type diterpenes have been isolated from the roots of *E. kansui* as described previously: 20-*O*-acetyl-[3-*O*-(2′*E*,4′*Z*)-decadienoyl]-



1: $R^1 = CO\text{-}\overset{E/Z}{(CH{=}CH)_2}\text{-}(CH_2)_4\text{-}CH_3$, $R^2 = H$, $R^3 = COCH_3$, $R^4 = H$

2: $R^1 = CO\text{-}\overset{E/Z}{(CH{=}CH)_2}\text{-}(CH_2)_4\text{-}CH_3$, $R^2 = H$, $R^3 = H$; $R^4 = H$

3: $R^1 = H$, $R^2 = CO\text{-}\overset{E/Z}{(CH{=}CH)_2}\text{-}(CH_2)_4\text{-}CH_3$, $R^3 = COCH_3$, $R^4 = H$

4: $R^1 = COCH(CH_3)CH(CH_3)_2$, $R^2 = H$, $R^3 = H$, $R^4 = OCO(CH_2)_{10}CH_3$

5: $R^1 = COCH(CH_3)CH(CH_3)_2$, $R^2 = H$, $R^3 = H$, $R^4 = OCO(CH_2)_8CH_3$

Fig. 1. Structure of the ingenol-type compounds **1** to **5**.

ingenol (**1**); 20-*O*-acetyl-[5-*O*-(2′*E*,4′*Z*)-decadienoyl]-ingenol (**2**); 3-*O*-(2′*E*,4′*Z*-decadienoyl)-ingenol (**3**) (Shi *et al.*, 2007); 3-*O*-(2″,3″-dimethylbutyryl)-13-*O*-dodecanoyl-ingenol (**4**); and 3-*O*-(2″,3″-dimethylbutyryl)-13-decanoyl-ingenol (**5**) (Shi *et al.*, 2008) (Fig. 1).

Kansuinin A (**6**): Colourless crystals; $[\alpha]_D^{22}$ +39.0° (*c* 0.2, MeOH). – UV (MeOH): λ_{max} (log ε) = 230 nm (4.21). – ESIMS: *m/z* = 748 $[M+NH_4]^+$. – IR (KBr): ν_{max} = 3456, 2974, 2942, 1725, 1657, 1605, 1586, 1377, 1234, 1036 cm^{-1}. – ^1H and ^{13}C NMR: see Table I.

Compound **7**: Colourless crystals; $[\alpha]_D^{22}$ +106.0° (*c* 0.2, MeOH). – UV (MeOH): λ_{max} (log ε) = 230 nm (4.08). – ESIMS: *m/z* = 688 $[M + NH_4]^+$. – IR (KBr): ν_{max} = 3650, 2978, 2942, 1742, 1688, 1649, 1605, 1586, 1375, 1272, 1236, 1025 cm^{-1}. – ^1H and ^{13}C NMR: see Table I.

Kansuinin B (**8**): Colourless crystals; $[\alpha]_D^{22}$ –3.0° (*c* 0.2, MeOH). – UV (MeOH): λ_{max} (log ε) = 231 (4.32). – ESIMS: *m/z* = 740 $[M + NH_4]^{+\cdot}$. – IR (KBr): ν_{max} = 3482, 2982, 2942, 1717, 1649, 1605, 1586, 1454, 1274, 1056, 1027 cm^{-1}. – ^1H and ^{13}C NMR: see Table II.

Compound **9**: Colourless crystals; $[\alpha]_D^{22}$ –37.0° (*c* 0.2, MeOH). – UV (MeOH): λ_{max} (log ε) = 228 nm (4.37). – ESIMS: *m/z* = 740 $[M + NH_4]^{+\cdot}$. – IR (KBr): ν_{max} = 3392, 2984, 2940, 1725, 1649, 1603, 1586, 1377, 1253, 1112, 1060 cm^{-1}. – ^1H and ^{13}C NMR: see Table II.

Results and Discussion

Compounds **6** and **8** are previously identified compounds, whose ^1H and ^{13}C NMR spectral data (Tables I and II) were identical with those of kansuinin A and kansuinin B, respectively (Uemura *et al.*, 1975a, b; Wang *et al.*, 2002; Pan *et al.*, 2004).

Compound **7** was obtained as colourless crystals, and the molecular formula of $C_{35}H_{42}O_{13}$ was indicated by ^1H NMR, ^{13}C NMR and MS spectral data. ^1H and ^{13}C NMR spectral data showed the presence of a benzoate group [δ_H 7.79 (2H), 7.54 (1H), 7.37 (2H); δ_C 133.5, 129.2 × 2, 129.2, 128.4 × 2, 164.6 (CO)] and four acetyl groups [δ_H 2.12 s, 2.10 s, 2.07 s, 1.85 s; δ_C 169.6, 169.5, 169.3, 170.6 (CO) and 21.5, 21.0, 20.9, 20.7 (CH_3)]. The correlation between H-11, H-13, Me-20 and the carbon signal at δ_C 204.7 revealed that one keto group was situated at C-12, and the correlation between H-1, H-4, H-13, H-20 and the carbon signal at δ_C 212.7 verified that the other keto group was placed at C-14. The ^{13}C NMR and DEPT spectral data indicated that the skeleton was composed of eight methyl groups, two methylene, fourteen methine groups, three quaternary carbon atoms and seven carbonyl groups. The correlations between H-11 and C-7, C-10, C-12, C-19, and between H-8, H-9, H-11, H-17 and C-7, in combination with the H-7 signal resonating at 4.38 (s) and moving upshift field by 2.01 ppm in comparison with that of kansuinin A (Table I), demonstrated that the oxygen bridge was at C-11 and C-7. The correlation of the benzoate carbonyl OCO-8 (δ_C 164.6) with H-8 (δ_H 5.57) demonstrated that the benzoyloxy group was located at C-8, and with the help of HMBC and HQBC spectral data, the positions of the acetoxy groups were also identified. In comparison with kansuinin A spectral data, the compound **7** was determined (Fig. 2) as a known compound (Uemura *et al.*, 1975a); however, the detailed NMR data of **7** were not reported by Uemura *et al.* (1975a), so we document the ^1H and ^{13}C NMR spectral data in Tables I and II.

Compound **9** was obtained as colourless crystals, and the molecular formula of $C_{38}H_{42}O_{14}$ was indicated by ^1H NMR, ^{13}C NMR and MS spectral data. ^1H and ^{13}C NMR spectral data exhibited the presence of two benzoate groups [δ_H 8.04 (2H),

Table I. ^{1}H and ^{13}C NMR data of compounds **6** and **7** [600 and 150 MHz, $CDCl_3$, δ (ppm)].

Position	**6**		Position	**7**	
	^{1}H	^{13}C		^{1}H	^{13}C
1	2.63 dd (13.8, 6.3), 2.28 m	40.0	1	2.57 dd (13.8, 6.0), 1.94 m	47.6
2	2.21 m	38.8	2	2.45 m	37.2
3	5.57 m	74.4	3	5.53 m	76.8
4	2.98 brs	51.4	4	3.31 dd (11.4, 4.8)	55.3
5	6.12 s	70.1	5	5.83 d (11.4)	73.3
6		145.4	6		139.5
7	6.39 s	68.7	7	4.38 d (10.8)	70.3
8	6.04 s	71.0	8	5.57 d (3.0)	64.7
9	5.05 s	82.4	9	5.26 d (3.0)	75.2
10		41.4	10		39.2
11	4.11 s	77.5	11	3.95 s	78.8
12		214.1	12		204.7
13	2.28 q (6.6)	50.9	13	3.58 q (6.6)	53.0
14		106.3	14		212.7
15		90.6	15		90.7
16	0.90 d (6.6)	13.3	16	0.91 d (6.6)	12.9
17	5.21 s, 5.12 s	106.3	17	5.59 d (2.4), 5.44 s	120.5
18	1.28 s	18.6	18	1.20 s	21.8
19	1.12 s	22.1	19	1.09 s	18.7
20	1.28 d (6.6)	9.1	20	1.25 d (6.6)	12.6
Acetyloxy			Acetyloxy		
3-OCO		169.5	3-OCO		169.6
3-OCOMe	1.98 s	22.0	3-OCOMe	2.10 s	21.5
5-OCO		168.8	5-OCO		169.3
5-OCOMe	1.93 s	20.9	5-OCOMe	1.85 s	21.0
7-OCO		170.5	9-OCO		169.5
7-OCOMe	2.16 s	21.1	9-OCOMe	2.12 s	20.7
9-OCO		169.2	15-OCO	170.6	
9-OCOMe	2.05 s	20.4	15-OCOMe	2.07 s	20.9
15-OCO		170.2	Benzoyloxy		
15-OCOMe	2.07 s	21.3	8-OCO		164.6
Benzoyloxy			8-OCOPh 1		133.5
8-OCO		165.4	2, 6	7.79 m	129.2
8-OCOPh 1		130.1	3, 5	7.37 m	128.4
2, 6	8.03 m	129.9	4	7.54 m	129.2
3, 5	7.41 m	128.3			
4	7.53 m	132.9			

7.82 (2H), 7.52 (1H), 7.44 (2H), 7.44 (1H), 7.32 (2H); δ_C 132.7, 132.5, 131.1, 130.0 × 2, 130.0, 129.4 × 2, 128.2 × 2, 128.0 × 2, 165.2 (CO), 164.5 (CO)] and two acetyl groups [δ_H 2.03 s, 1.85 s; δ_C 173.0, 170.0 (CO) and 21.6, 21.1 (CH_3)]. The correlation between H-8, Me-18, Me-19 and the carbon signal at δ_C 214.5 demonstrated that one keto group was situated at C-9; the correlation between H-1, H-4, H-20 and the carbon signal at δ_C 204.8 confirmed that the other keto group was placed at C-14. Additionally, the correlation of the benzoate carbonyl OCO-5 (δ_C 164.5) with H-5 (δ_H 5.93) demonstrated that one benzoyloxy group was located at C-5, and the attachments of the C-7 benzoyloxyl group, the C-3 acetoxy group, and the C-15 acetoxy group were determined in the same manner with the help of HMBC and HMQC. The features of the ^{1}H NMR spectral data showed that the H-8, H-13 and H-17 signals of compound **9** apparently shifted upfield, and that the H-12 signal moved downfield compared with those of kansuinin B. This demonstrated that the oxygen bridge may be situated at C-11 and C-8, and a hydroxy group at C-12. Moreover, the correlation between H-11 and C-7, C-10 and C-12 further ascertained the attachment of the oxygen bridge. Therefore, compound **9** was ascertained (Fig. 2) as a known compound (Uemura *et al.*, 1975b); however, the explicit NMR data of **9** were not reported by Uemura *et al.* (1975b). Consequently, we docu-

Table II. ^{1}H and ^{13}C NMR data of compounds **8** and **9** [600 and 150 MHz, $CDCl_3$, δ (ppm)].

Position	**8**		**9**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	4.31 s	87.1	4.25 s	85.1
2		78.3		79.3
3	5.51 d (4.8)	76.8	5.75 d (4.2)	76.0
4	3.61 dd (4.8, 11.4)	45.0	4.33 m	47.4
5	5.89 m[a]	73.8	5.93 s	66.5
6		135.8		144.2
7	5.89 m[a]	64.6	5.95 d (1.2)	73.1
8	4.65 s	72.7	3.93 d (1.2)	82.9
9		209.5		214.5
10		48.1		47.0
11	3.68 d (1.2)	60.8	3.55 d (9.6)	87.1
12	3.29 d (2.4, 9.6)	59.0	4.38 m	72.7
13	4.11 m	42.5	3.42 m	47.5
14		204.9		204.8
15		96.1		94.9
16	1.27 s	20.1	1.35 s	20.3
17	6.50 brs	128.3	6.12 d (15.6)	120.9
18	1.33 s	21.6	1.16 s	21.7
19	0.83 s	19.0	1.02 s	17.8
20	1.51 d (6.0)	17.0	1.32 d (6.6)	15.3
Acetyloxy				
3-OCO		168.8		170.0
3-OCOMe	1.88 s	20.4	2.03 s	21.6
15-OCO		172.4		173.0
15-OCOMe	2.15 s	21.3	1.85 s	21.1
Benzoyloxy				
5-OCO		164.9		164.5
5-COPh 1		128.3		131.1
2, 6	7.52 d (7.8)	129.2	7.82 d (7.8)	129.4
3, 5	7.01 t (7.8)	127.7	7.32 t (7.8)	128.0
4	7.22 m	132.8	7.44 m[a]	132.5
7-OCO		166.0		165.2
7-OCOPh 1		128.3		130.0
2, 6	7.48 d (7.8)	129.4	8.04 d (7.8)	130.0
3, 5	6.91 t (7.8)	127.8	7.44 m[a]	128.2
4	7.08 m	132.8	7.52 t (7.8)	132.7
OH-1	4.09 d (7.2)		2.37 brs	
OH-2	2.30 s		2.30 s	
OH-8	3.40 m		2.17 s[b]	

[a] Overlapping signal.

[b] OH-12.

ment the ^{1}H and ^{13}C NMR spectral data in Tables I and II.

The compounds were assessed for activity against the termite *R. speratus* at 50, 25 and 12.5 μg/disk, respectively, compared with the untreated control. The percent mortalities obtained showed that compounds **1** to **5** produced more than 83% mortality 36 h after treatment at 50 μg/disk, and that the remaining compounds were significantly different from the untreated controls at the same content indicating that they were slightly active. At this time interval, compound **5** displayed the highest activity, producing a mortality of 96.43% (Table III). The results obtained with compounds **1** to **5** tested 48 h after treatment were similar to those at 36 h after treatment, and showed much higher activity than the remaining compounds. 60 h after treatment, compounds **1** to **5** remained the most active of the compounds tested, producing termite mortalities of 97.32% or 100%, respectively, and the remaing compounds also appeared to be active based on the finding of no significant difference from the untreated control (Table III). After 72 h of testing the mortality rate induced by compounds **1** to **5** reached 100%, and the remaining compounds exhibited slight activity indicated by a significant difference from the untreated control.

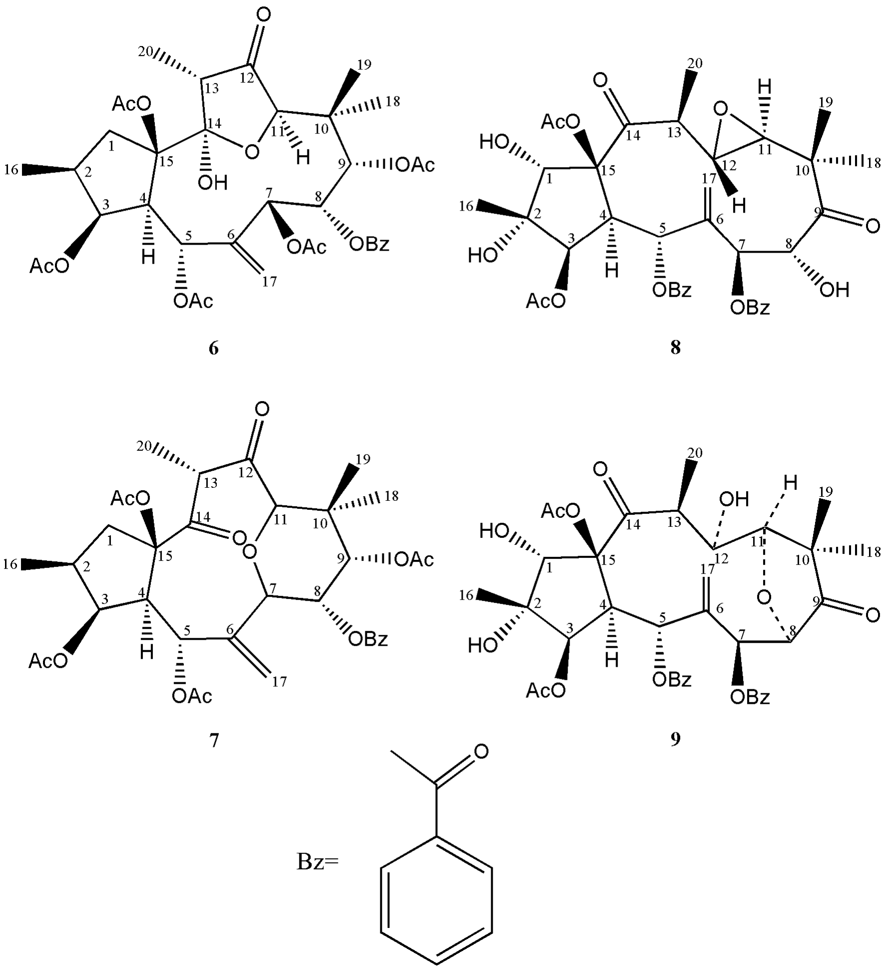


Fig. 2. Structure of kansuinins A (**6**) and B (**8**) and of their derivatives **7** and **9**.

At 25 μg/disk compounds **1** to **5** had the strongest termiticidal activity observed at any time, and gave 100% termiticidal activity after 72 h. Apart from compounds **1** to **5**, the remaining compounds appeared slightly active compared to the control.

The termiticidal activities of compounds **1** to **5** tested at 12.5 μg/disk at 36 h after treatment were higher than those of the remaining compounds (Table III). At 48 h after treatment, the highest mortalities were observed with compounds **2** [(92.86 ± 2.84)%], **3** [(90.18 ± 2.78)%], and **5** [(80.36 ± 5.56)%], followed by compounds **4** [(73.21 ± 5.29)%] and **1** [(72.22 ± 1.74)%]. The remaining compounds showed slight activity in comparison with the untreated control at 48 h. Compounds **2** to **5** showed mortalities of 100%, and the mortality produced by compound **1** was (93.06 ± 5.56)% at 72 h after treatment (Table III).

Toxicity and repellency are two important factors in treating termites infesting wooden structures and cellulosic materials (Maistrello *et al.*, 2001). During the course of the test, we observed that the termites consumed not only the untreated paper disks but also the treated paper disks. The termites that ingested the treated paper disks containing compounds **1** to **5** appeared to have the highest mortality compared with the termites consuming untreated paper disks. We also observed that the termites that consumed the treated papers died very rapidly. It is evident that compounds **1** to **5**, which have the ingenol-type feature, showed

Table III. Cumulative percent mortality of *Reticulitermes speratus* in the vial test.

Compound	Content per disk [μg]	% Mortality (mean ± SE)[a]			
		36 h	48 h	60 h	72 h
1	50	83.048 ± 2.50b	91.96 ± 2.71a	97.32 ± 1.47a	100 ± 0a
2		93.762 ± 2.28a	99.11 ± 0.69a	100 ± 0a	100 ± 0a
3		88.40 ± 3.77a	93.75 ± 2.02a	97.32± 1.04a	100 ± 0a
4		94.64 ± 3.13a	96.43 ± 2.11a	100 ± 0a	100 ± 0a
5		96.43 ± 1.39a	98.21 ± 0.92a	100 ± 0a	100 ± 0a
6		8.04 ± 2.55c	13.40 ± 2.20b	14.29 ± 2.02b	23.21 ± 3.47b
7		10.71 ± 3.13c	10.71 ± 3.13b	15.18 ± 4.22b	23.21 ± 6.11b
8		9.82 ± 2.08c	13.39 ± 1.63b	16.96 ± 2.33b	25.89 ± 4.77b
9		8.93 ± 2.35c	15.18 ± 4.03b	20.54 ± 5.78b	23.21 ± 4.93b
Untreated	0	0 ± 0d	0 ± 0c	0 ± 0c	0 ± 0c
1	25	77.68 ± 4.72b	90.18 ± 2.97a	96.43 ± 2.08a	100 ± 0a
2		91.07 ± 3.09a	97.32 ± 1.44a	99.11 ± 0.69a	100 ± 0a
3		76.79 ± 5.21b	83.93 ± 4.09b	90.18 ± 2.55b	100 ± 0a
4		91.07 ± 4.00a	95.54 ± 1.80a	100 ± 0a	100 ± 0a
5		79.46 ± 2.76b	87.50 ± 2.35b	100 ± 0a	100 ± 0a
6		5.36 ± 1.93c	6.25 ± 3.89c	7.14 ± 5.87d	19.44 ± 6.54b
7		6.25 ± 1.80c	7.64 ± 2.50c	9.82 ± 3.31c	11.81 ± 3.37c
8		4.17 ± 1.40c	5.56 ± 1.63c	12.5 ± 1.46c	15.97 ± 2.35b
9		4.17 ± 1.80c	6.25 ± 1.80c	6.94 ± 1.63d	9.72 ± 2.78c
Untreated	0	0 ± 0d	0 ± 0d	0 ± 0e	0 ± 0d
1	12.5	64.587 ± 12.50b	72.22 ± 1.74b	83.33 ± 7.37b	93.06 ± 5.56b
2		88.40 ± 3.29a	92.86 ± 2.84a	98.21 ± 0.92a	100 ± 0a
3		78.57 ± 2.57a	90.18 ± 2.78a	90.18 ± 2.91a	100 ± 0a
4		63.39 ± 7.51b	73.21 ± 5.29b	90.97 ± 3.91a	100 ± 0a
5		75.00 ± 4.18a	80.36 ± 5.56a	96.53 ± 1.83a	100 ± 0a
6		2.68 ± 1.47c	2.68 ± 1.47c	3.57 ± 1.51c	4.86 ± 1.74c
7		4.17 ± 2.33c	6.94 ± 2.84c	9.72 ± 3.47c	10.42 ± 3.29c
8		2.78 ± 1.51c	5.56 ± 1.93c	9.72 ± 2.13c	10.42 ± 3.29c
9		0.69 ± 0.69c	2.78 ± 1.51c	6.25 ± 2.55c	6.94 ± 2.43c
Untreated	0	0 ± 0c	0 ± 0c	0 ± 0d	0 ± 0d

Means within a column of each toxicant with the same letter are not significantly different (Tukey's test, $P < 0.05$).

[a] 16 worker termites per replicate with nine replicates.

the highest termiticidal activity against the termite *R. speratus*, whereas compounds **6** to **9** exhibited slight or no activity.

The roots of *E. kansui*, when used as a Chinese traditional medicine, have a significant function in treating some diseases (Pharmacopoeia Commission, 1995; Xu *et al.*, 1997; Xia and Li, 1999). Therefore, many active compounds have been identified and isolated from the plant. In particular, many ingenane compounds have been obtained, and these show inhibition of cell proliferation and topoisomerase II activity. It has been reported that ingenol-3-esters exhibit irritant and tumour-promoting activities, whereas ingenol-20-esters appeared to be inactive (Opferkuch and Hecker, 1982). It has also been observed that the C-20 ester has reduced tumour-promoting activities, but has retained its antileukemic activity (Wu *et al.*, 1991).

At 72 h after treatment, the assay showed little difference in the termiticidal activity of compounds **1**, **2**, **4** and **5** (ingenol diester derivatives) and compound **3** (ingenol monoester derivatives). Therefore, if the ingenane compounds possessing tumour-promoting activity were esterified at C-20 retaining their termiticidal activity, they could potentially be used for pest control application to replace synthetic pesticides that can persist in the environment and result in many unpredictable problems (Isman, 2006).

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