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INSECTICIDAL CONSTITUENTS FROM RHIZOMES OF *ZINGIBER CASSUMUNAR* AND *KAEMPFERIA ROTUNDA*

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Key Word Index—*Kaempferia rotunda*; *Zingiber cassumunar*; Zingiberaceae; rhizomes; insecticidal constituents; *Spodoptera littoralis*.

Abstract—Rhizomes from 18 different species of the Zingiberaceae were screened for insecticidal constituents against neonate larvae of the pest insect, *Spodoptera littoralis*. Extracts from rhizomes of *Kaempferia rotunda* and *Zingiber cassumunar*, when incorporated into artificial diets, displayed significant insecticidal activity in chronic feeding bioassays at concentrations of 2500 ppm and 1250 ppm, respectively. Bioassay-guided isolation afforded two phenylbutanoids from rhizomes of *Z. cassumunar* which had LC_{50} values against neonate larvae of 121 and 127 ppm, respectively, in the chronic feeding bioassay. Both compounds were also active in the residue-contact bioassay (LC_{50} values of 0.5 and 3.6 $\mu g\,cm^{-2}$, respectively). The presence of oxygenated substituents (–OH or –OAc groups) in the side-chain nullified insecticidal activity. Rhizomes of *K. rotunda* yielded two active metabolites: benzyl benzoate and the cyclohexane derivative, crotepoxide. Compared to the bioactive phenylbutanoids from *Z. cassumunar*, crotepoxide was less active in the chronic feeding bioassay (LC_{50}, 1450 ppm) and was inactive in the residue-contact bioassay. Benzyl benzoate, however, exhibited insecticidal activity only when applied topically (LC_{50}, 5.6 $\mu g\,cm^{-2}$) suggesting detoxification in the larval gut when applied orally.

INTRODUCTION

Members of the Zingiberaceae are famous for their use as spices or as medicinal herbs. Well-known examples include the rhizomes of *Zingiber officinale* (ginger) or *Curcuma longa* (syn. *C. domestica*) (turmeric) [1–3]. Rhizomes of *C. longa* or of *Kaempferia pandurata* are important in the folk medicine of South East Asia as antiseptics for wounds or as expectorants [4–8]. Rhizomes of several species from the Zingiberaceae, however, also contain insecticidal constituents. Dried and powdered rhizomes of *C. longa*, for example, have been reported to deter storage-pest insects, such as *Tribolium castaneum* [9]. Recently, we could show that the sesquiterpene, xanthorrhizol, as well as other sesquiterpenes that are present in rhizomes of *Curcuma xanthorrhiza* or *C. zedoaria*, are insecticidal towards larvae of the vigorous pest insect, *Spodoptera littoralis*, when applied topically via the larval integument [10].

We have now screened crude extracts from rhizomes of 19 different species of the Zingiberaceae and report on the insecticidal constituents isolated from *Z. cassumunar and K. rotunda*, which were the most active species analysed.

RESULTS AND DISCUSSION

Extracts from rhizomes of 18 different species of the Zingiberaceae were incorporated into artificial diets at two arbitrarily chosen concentrations (1250 and 2500 ppm) and analysed for insecticidal activity towards neonate larvae of *S. littoralis*. After six days of exposure to the treated diet, larval survival and weight were monitored and compared to controls (Table 1). Rhizome extracts of *K. rotunda* L. and of *Z. cassumunar* Roxb. exhibited significant insecticidal activity and caused strong larval mortality and/or reduction of larval weight. The extract of *Z. cassumunar* was more active than that of *K. rotunda* resulting in complete larval mortality even at the lowest concentration analysed (1250 ppm), whereas a significant reduction of larval survival by the extract from *K. rotunda* was only observed at the highest concentration analysed (Table 1).

Bioassay-guided isolation of the rhizomes of *Z. cassumunar* afforded two phenylbutanoid derivatives (**1** and **2**, Fig. 1) which were shown to be responsible for the

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Table 1. Survival and growth of neonate larvae of *Spodoptera littoralis* following chronic exposure to artificial diet spiked with crude extracts from rhizomes of same Zingiberaceae*

Species	Dose (in ppm)	Survival (in % relative to (controls)	Weight (in % relative to (controls)
Aframomum melegueta K. Schum	1250	100	67
	2500	90	40
Alpinia galanga Sw.	1250	100	76
	2500	95	46
A. malaccensis Rosc.	1250	100	90
	2500	90	58
Amomum cardamomum Willd	1250	100	81
	2500	100	69
Costus ananassae Hassk.	1250	100	100
	2500	100	64
C. igneus (Nees et Martius) Mass.	1250	100	99
	2500	95	65
Curcuma aeruginosa Roxb.	1250	90	24
	2500	80	12
C. domestica Val.	1250	90	51
	2500	90	28
C. heyneana Val. & V. Zyp.	1250	100	46
	2500	80	17
C. purpurascens Bl.	1250	95	73
	2500	100	27
C. xanthorrhiza Roxb.	1250	100	47
	2500	95	35
C. zedoaria Rosc.	1250	90	39
	2500	75	18
Kaempferia galanga L.	1250	100	38
	2500	95	38
K. pandurata Roxb.	1250	90	93
	2500	100	70
K. rotunda Linn.	1250	78	4
	2500	23	2
Zingiber aromaticum Val.	1250	90	79
	2500	100	69
Z. cassumunar Roxb.	1250	0	0
	2500	0	0
Z. officinale Rosc.	1250	100	77
	2500	100	56

*Neonate larvae of *S. littoralis* ($n = 20$) were released on the treated diet. After 6 days of exposure, survival and weight of the surviving larvae were measured and compared to controls that had been exposed to diet treated with solvent (acetone) only. Mortality of control larvae was usually in the range of 0–5% during the experiment. Weight of control larvae at the end of the experiment varied from 40–50 mg larva^{-1}. Data reported are means of three independent experiments.

insecticidal activity of the crude extract (Table 2). Both compounds were identified from their NMR and mass spectra and by comparison with previously published data [11]. When incorporated into artificial diet and offered to neonate larvae of *S. littoralis* in a chronic feeding experiment over a period of six days, compounds **1** and **2** (Fig. 1) were similar with regard to insecticidal activity. The LC_{50} values of **1** and **2** were 121 and 127 ppm (0.63 and 0.57 μmol g^{-1} fr. wt of diet), respectively, whereas the EC_{50} values were 41 and 36 ppm (0.22 and 0.16 μmol g^{-1} fr. wt of diet), respectively (Table 2). Both phenylbutanoids (**1** and **2**) were also active against neonate larvae of *S. littoralis* when applied topically via the larval integument. Each compound was tested for contact toxicity using coated glass vials at a range of concentrations (0.06–13 μg cm^{-2}) [12]. The LC_{50} values of both compounds (**1** and **2**) were 0.5 and 3.6 μg cm^{-2}, respectively (Table 2). Both phenylbutanoid metabolites (**1** and **2**) had been isolated previously [11], although their insecticidal properties have not been described.

The contact toxicity of phenylbutanoid **1** towards neonate larvae of *S. littoralis* was comparable to that of the sesquiterpene, xanthorrhizol, (LC_{50}, 0.3 μg cm^{-2}) which was recently described as a natural insecticide

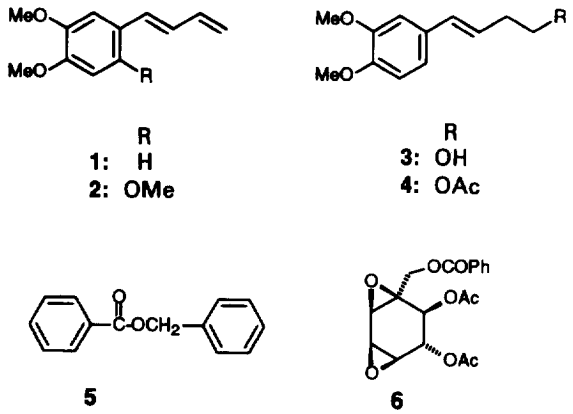

Fig. 1. Compounds isolated from rhizomes of *Zingiber cassumunar* (**1–4**) and *Kaempferia rotunda* (**5** and **6**).

present in rhizomes of *C. xanthorrhiza* [10]. In contrast with compound **1**, however, which is also active upon oral administration, xanthorrhizol exhibited only marginal insecticidal activity when incorporated into artificial diets which was proposed to be due to detoxification in the larval gut [10].

In addition to compounds **1** and **2**, two further phenylbutanoids (**3** and **4**) (Fig. 1) were isolated from rhizomes of *Z. cassumunar* and identified by their NMR and mass spectra, as well as by comparison with published data [11, 13]. Compounds **3** and **4** differed from **1** and **2** by the absence of one of the side-chain double bands, as well as by the presence of terminal oxygen substituents on the butanoid side-chains (–OAc or –OH groups, respectively). Compounds **3** and **4** were devoid of insecticidal activity in the chronic feeding bioassay or contact-residue bioassay at the range of concentrations tested (Table 2). This may be ascribed to the presence of polar substituents (–OH or –OAc groups in **3** and **4** as compared to alkyl substituents in **1** or **2**) that increase polarity and thereby facilitate excretion via the faeces and/or reduction of the conjugated system in **3** and **4** compared to that of **1** or **2**.

Two compounds with insecticidal activity were isolated from rhizomes of *K. rotunda*. Benzyl benzoate (**5**) (Fig. 1) exhibited insecticidal activity towards neonate larvae of *S. littoralis* only when applied topically via the larval integument. The LC_{50} of compound **5** was observed at 5.6 $\mu g\,cm^{-2}$ in the glass vial assay (Table 2). Benzyl benzoate, however, was inactive when administered orally (Table 2). The major compound with insecticidal properties present in rhizomes of *K. rotunda* was identified as the cyclohexane oxide derivative, crotepoxide (**6**) (Fig. 1) originally isolated from *Croton macrostachys* (Euphorbiaceae) [14]. Crotepoxide was identified by its NMR and mass spectra, as well as from a comparison with previously published data [14]. Following exposure of neonate larvae of *S. littoralis* to artificial diets spiked with various concentrations of **6**, the LC_{50} and EC_{50} of crotepoxide were 1450 ppm (4 $\mu mol\,g^{-1}$ fr. wt of

Table 2. LC_{50} and EC_{50} values of insecticidal constituents isolated from rhizomes of *Kaempferia rotunda* (**5**, **6**) and *Zingiber cassumunar* (**1**, **2**) towards neonate larvae of *Spodoptera littoralis*

	Chronic feeding experiment*				Contact toxicity†	
	LC_{50}		EC_{50}		LC_{50}	
Compound	ppm	$\mu mol\,g^{-1}$ fr. wt	ppm	$\mu mol\,g^{-1}$ fr. wt	$\mu g\,cm^{-2}$	$\mu mol\,cm^{-2}$
1	121	0.63	41	0.22	0.5	0.0026
2	127	0.57	36	0.16	3.6	0.016
5	NA	NA	NA	NA	5.6	0.026
6	1450	4.0	70	0.2	NA	NA

*Chronic feeding experiment: Neonate larvae of *S. littoralis* ($n = 20$) were released on diet spiked with various concentrations of the analysed compounds (10–2000 ppm). After 6 days of exposure, survival and weight of the surviving larvae were measured and compared to controls that had been exposed to diet treated with solvent (acetone) only. From the dose–response curves (means of three independent experiments) obtained, the respective LC_{50} and EC_{50} values were calculated by probit analysis.

†Contact toxicity: Compounds studied were coated as an even film on the inside of glass vials in a series of concentrations ranging from 0.05–1.0 $\mu mol\,vial^{-1}$. After evaporation of carrier (acetone) 25 neonate larvae were placed inside the vials. Vials were covered with cotton wool to prevent the larvae from escaping. After an initial period of 3 hr, food was added to the vials. After 48 hr, mortality of larvae was monitored and compared to controls that were kept in vials treated with acetone only. From the dose–response curves (means of three independent experiments) obtained, LC_{50} values were calculated by probit analysis. Compound **3** and **4** were inactive both in the chronic feeding as well as in the contact-toxicity experiment. Numbers of compounds refer to Fig. 1.

‡NA: not active at concentrations analysed.

diet) and 70 ppm (0.2 μmol g^{-1} fr. wt of diet), respectively (Table 2). Crotepoxide was thus significantly less active towards larvae of *S. littoralis* than the phenylbutanoids **1** or **2** (Table 2) which accounts for the inferior insecticidal activity of the crude extracts from rhizomes of *K. rotunda* compared with those of *Z. cassumunar* (Table 1). No insecticidal activity of **6** was observed upon topical application to the larvae using coated glass vials (Table 2). The tumour inhibiting activity of crotepoxide has been described [14]. However, this is the first report of the insecticidal properties of this unusual natural product.

EXPERIMENTAL

Isolation and spectroscopic identification of compounds. Extracts were obtained from fr. rhizomes supplied by the Institut Pertanian Bogor (Indonesia). Rhizomes were cut and exhaustively extracted with Me_2CO with stirring at room temp. Following filtration, the Me_2CO extracts were concd under red. pres. and subsequently partitioned between H_2O and EtOAc. The EtOAc-soluble extracts were used for the initial screening expt for insecticidal activity (Table 1). For isolation of pure compounds the EtOAc extracts were sepd by CC on silica gel (employing mixts of CH_2Cl_2 and MeOH or of hexane and Et_2O as mobile phase), Sephadex LH-20 (MeOH or Me_2CO as mobile phase) or on RP-18 (mixts of MeOH and H_2O as mobile phase). Each fractionation was monitored by bioassays employing larvae of *S. littoralis*. ^1H and ^{13}C NMR spectra were recorded on Bruker AM 300 or ARX 400 NMR spectrometers. MS were obtained by GC-MS or by direct inlet. GC-MS: GC equipped with a 30 m × 0.32 mm id fused silica capillary column coated with DB-1, temp. prog. 100–300° at 6° min^{-1}. The structure of **1–6** were readily determined from 1D (^1H and ^{13}C) and 2D (COSY, ^1H-detected direct, and long-range ^{13}C–^1H correlations) and by comparison with published data [11, 13, 14].

Experiments with insects. Larvae of *S. littoralis* were from a laboratory colony reared on artificial diet under controlled conditions as described previously [12]. Contact toxicity was studied by a residue contact bioassay employing neonate larvae. A known dose of compound was dissolved in 400 μl of Me_2CO and introduced into glass vials. Each vial was rotated by hand until the soln was evenly distributed on the inner wall and base, thereby allowing the solvent to evaporate. After 1 hr, neonate larvae (n = 25) were placed into the vials. Insects were allowed free movement for 3 hr in the closed vials. During this time, the vials contained no food. After the initial contact of 3 hr, diet was added. After 48 hr survival of larvae was recorded. Feeding studies were conducted with neonate larvae ($n = 20$) that were kept on an artificial diet with the extracts, frs or compounds under study. After 6 days, survival and wt of larvae were recorded and compared with controls.

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REFERENCES

1. Brücher, H. (1977) *Tropische Nutzpflanzen. Ursprung, Evolution und Domestikation.* Springer, Berlin.
2. Simmonds, N. W. (1976) *Evolution of Crop Plants.* Longman, London.
3. Purseglove, J. W. (1972) *Tropical Crops, Monocotyledons.* Longman, London.
4. Rose, M. F. S. and Brain, K. R. (1977) in *An Introduction to Phytopharmacy*, p. 159. Pittman Medical, Tunbridge Wells, U.K.
5. Evans, B. K., James, K. C. and Luscombe, D. K. (1978) *J. Pharmacol. Sci.* **67**, 277.
6. Haginiwa, J., Harada, M. and Merishita, I. (1963) *Yakugaku Zasshi* **83**, 624.
7. Bunyapraphatsara, N. (1990) in *Economic and Medicinal Plant Research* (Wagner, H. and Farnsworth, N. R., eds), Vol. 4, p. 141. Academic Press, London.
8. Tuntiwachwuttikul, P., Pancharoen, O., Reutrakul, V. and Byrne, L. T. (1984) *Aust. J. Chem.* **37**, 449.
9. Grainge, M. and Ahmed, S. (1988) *Handbook of Plants with Pest-Control Properties.* John Wiley, New York.
10. Pandji, C., Grimm, C., Wray, V., Witte, L. and Proksch, P. (1993) *Phytochemistry* **34**, 415.
11. Tuntiwachwuttikul, P., Pancharoen, O., Jaipetch, T. and Reutrakul, V. (1981) *Phytochemistry* **20**, 1164.
12. Srivastava, R. P. and Proksch, P. (1991) *Entomol. Gener.* **15**, 265.
13. Kuroyanagi, M., Fukushima, S., Yoshihira, K., Natori, S., Dechatiwongse, T., Mihashi, K., Nishi, M. and Hara, S. (1980) *Chem. Pharmacol. Bull.* **28**, 2948.
14. Kupchan, S. M., Hemingway, R. J. and Smith, R. M. (1969) *J. Org. Chem.* **34**, 3898.