

Larva-Development Inhibitors of Black Pepper

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Three inhibitors of drosophila larva-development have been isolated from black pepper, dried fruits of *Piper nigrum* L. By spectral evidence, they were identified as piperonal(**1**), piperine(**2**) and piperolein B(**3**). Besides these compounds, (2*E*, 4*E*)-*N*-isobutyl-2, 4-decadienamamide (**4**), known to be an insecticidal substance, was also isolated. It was, however, inactive against the larvae.

KEY WORDS: Larva-development inhibitor/ Black pepper/ Piper nigrum L./ Piperonal/ Piperine/ Piperolein B/

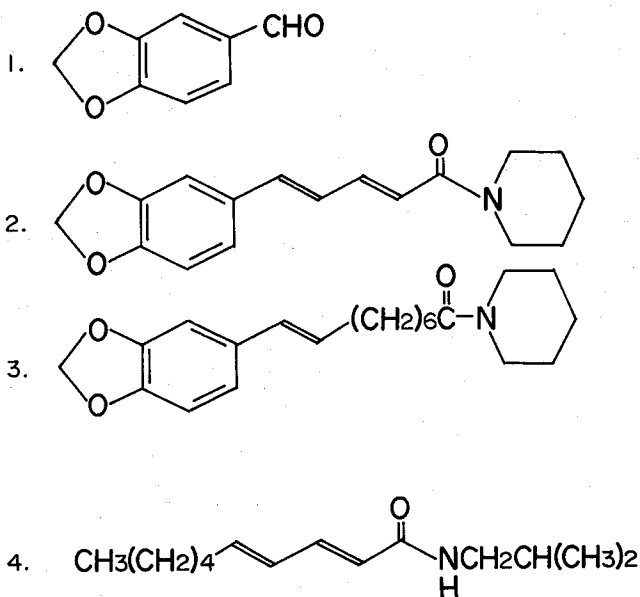
In the course of searching for insecticidal properties of several plant-extracts by using a convenient assay with drosophila larvae,¹⁾ we found that the extracts of several kinds of spices showed the inhibitory activity of the larva-development. Among them, the methanol extract of black pepper showed a remarkable activity.²⁾ This report is concerned with the isolation, identification of the active compounds and their activity.

Isolation of the active compounds was guided by the assay previously reported.¹⁾ Black pepper was extracted with *n*-hexane and ethyl acetate successively. Both extracts were highly active against one or two days old larvae. Purification of each extract by column chromatography on silica gel gave compound **1** from the *n*-hexane extract, and **2** and **3** from the ethyl acetate extract.

The physicochemical data of compound **1** and **2** were as follows: **1**, colorless prisms, mp 37°C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ), 230 (17,500), 272 (7700), 312 (9300), IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹, 3060, 2990, 2850, 1680, 1605, 1500, 1455, 1265, 1040, 865, 815, 785, ¹H-NMR(CDCl₃) δ , 6.01 (2H, s), 6.87 (1H, d, *J*=8 Hz), 7.19 (1H, d, *J*=1 Hz), 7.37 (1H, dd, *J*=8 and 1 Hz), 9.77 (1H, s), and **2**, pale yellow prisms, mp 129~130°C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ), 240 (13,900), 308 (sh., 18,500), 333 (23,600), IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹, 3000, 2040, 1640, 1620, 1590, 1500, 1450, 1260, 1030, 850, 830, 807, ¹H-NMR (CDCl₃) δ , 1.63 (6H, br.), 3.54 (4H, br.), 5.90 (2H, s), 6.34 (1H, d, *J*=14 Hz), 6.6~6.9 (5H, complicated), ca. 7.3 (1H, m). Direct comparison of the spectral data with those of authentic samples revealed that **1** is piperonal, known as a pediculicide,³⁾ and **2** is piperine, a pungent principle of the pepper.³⁾

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Compound **3**, a colorless viscous matter, showed the following spectral data: MS m/z , 343 (M^+ , $C_{21}H_{29}NO_3$), 127 (base peak), UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ), 260 (10,500), 268 (10,500), 303 (4400), $\text{IR}_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} , 2780, 1635, 1500, 1040, 970, and $^1\text{H-NMR}$ (CDCl_3) δ , 1.3~1.8 (14H), 2.0~2.5 (4H), 3.3~3.7 (4H), 5.89 (2H, s), 5.97 (1H, dt, $J=16$ and 6 Hz), 6.28 (1H, d, $J=16$ Hz), 6.70 (2H, s), 6.84 (1H, s). These spectral evidence suggested that **3** might be piperolein B, which has been isolated as a minor constituent of black pepper.⁴⁾ The identity was confirmed by comparison of the data with those of piperolein B, reported by Grewe *et al.*⁴⁾ This is the first finding that piperolein B possesses the inhibitory activity of the larva-development.

The inhibitory effects of **1**, **2** and **3** on the larva-development were tested against one or two days old larvae (Experiment-I) and four or five days old larvae (Experiment-II), and the results are summarized in Table I. **1**, **2** and **3** are active especially against the larvae at juvenile stage. Although piperine (**2**) has been occasionally used as an insecticide,³⁾ its pungency has reduced the usefulness. Here the effectiveness of piperolein B (**3**) as an insecticide is suggested because of its non-pungency and odorless.

Besides these compounds, we isolated (2*E*, 4*E*)-*N*-isobutyl-2, 4-decadienamide (**4**),** which has been known to be an insecticidal principle of pellitorine mixture obtained from the extract of *Anacyclus pyrethrum* D.C.⁵⁾ **4** was, however, inactive against the larvae at the concentrations tested, as shown in Table I.

As many compounds with a methylenedioxyphenyl group has been known as synergists to pyrethrins,⁷⁾ we tested the activity of piperonyl butoxide,⁸⁾ the most potent synergist so far known. As listed in Table I, it showed very high inhibitory activity of the larva-development.

Throughout the experiments, it has been suggested that a methylenedioxyphenyl group may be an important function for the inhibition of the larva-development.

** The structure of **4** was synthetically confirmed.⁶⁾

Table I. Inhibitory Effect of the Compounds on Larva-Development

Compound	Dosage mg/ml medium	No. of larvae treated	No. of pupae formed	No. of adults emerged
Piperonal (1)				
Experiment-I	10	10	6 ^a	1
	5	10	3 ^a	0
	1	10	6 ^a	1
Experiment-II	10	10	8	8
Piperine (2)				
Experiment-I	5	10	1	0
	1	10	3 ^a	1
	0.5	10	3 ^a	0
	0.1	10	8	6
Experiment-II	10	10	9	7
	5	10	9	9
Piperolein B(3)				
Experiment-I	10	10	0	0
	5	10	2 ^a	0
	1	10	1 ^a	0
Experiment-II	6	10	1 ^a	1
	2	10	5	5
	0.5	10	10	9
(2E, 4E)-N-isobutyl- 2, 4-decadienamide(4)				
Experiment-I	10	10	10	10
Experiment-II	10	10	10	10
Piperonyl butoxide				
Experiment-I	0.1	10	0	0
	0.05	10	4	4
Experiment-II	0.5	10	0	0
	0.1	10	10	8
Control				
Experiment-I		10	10	10
Experiment-II		10	10	10

a. The pupae were smaller than those of the control experiments.

EXPERIMENTAL

Melting points were determined on a hot stage and are uncorrected. ¹H-NMR spectra were obtained on a Hitachi Model R-22 spectrometer (90 MHz). In the spectra, chemical shifts are expressed in ppm from tetramethylsilane as an internal standard and singlet, doublet, triplet, double doublet, double triplet and multiplet are abbreviated to s, d, t, dd, dt and m, respectively. IR and UV spectra were recorded on a Hitachi EPI-G3 infrared spectrometer and Shimadzu UV-200 spectrometer,

respectively. MS spectra were obtained by a Hitachi RMU-6L mass spectrometer at 70 eV with EI ionization. The following chromatographic materials were used: Wako gel C-100 (Wako Pure Chemical Industries Ltd.), silicic acid (100 mesh, Mallinckrodt, U. S. A.) and silica gel H (E. Merck Co.) for column chromatography. Silica gel GF₂₅₄ (E. Merck Co.) and silica gel 60 PF₂₅₄ (E. Merck Co.) for thin layer chromatography (TLC) and preparative TLC, respectively.

Bioassay. Bioassay was performed by the method previously reported.¹⁾ In this experiment, the medium for cultivation of the larvae was changed from the previous method to Ohba's medium.⁹⁾

Isolation of 1, 2, 3 and 4. Black pepper (1 kg), dried fruits of *P. nigrum*, was extracted with *n*-hexane (31) for two weeks at room temperature. The solvent was removed off under reduced pressure to give an yellow oil (22.6 g). The oil (22 g) was chromatographed on Wako gel (500 g) with *n*-hexane containing an increasing amount of benzene. An active fraction, benzene eluate, was further chromatographed on silica gel H and eluted with a solvent (*n*-hexane-benzene, 1:1) under pressure. The combined active fraction was further purified by preparative TLC developed with a solvent (*n*-hexane-acetone, 5:2) to give a crystalline matter. Recrystallization from benzene-*n*-hexane gave **1** (12 mg) as colorless prisms.

The fruits after extraction with *n*-hexane were further extracted with ethyl acetate (31) for another two weeks. Evaporation of the solvent gave a dark yellow viscous matter (92 g) which was chromatographed on Wako gel (500 g) and eluted with benzene containing an increasing amount of ethyl acetate. The activity was found in the fractions eluted with 20 and 30% ethyl acetate in benzene. The combined fraction gave a crystalline material. Filtration and recrystallization from ethanol yielded piperine (**2**) (2.8 g) as pale yellow prisms. The filtrate was further chromatographed on silicic acid and eluted with 30% ethyl acetate in *n*-hexane to give an active fraction-A, which was purified on silica gel H eluted with 40% ethyl acetate in *n*-hexane to give **3** (130 mg) as a colorless viscous matter.

From the fraction-A, inactive compound **4** was also isolated as colorless needles, mp 89°C. MS *m/z*: 223 (M^+ , C₁₄H₂₅NO), UV $\lambda_{\text{Max}}^{\text{MeOH}}$ nm (ϵ): 257 (30,000), IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3450, 1675, 1640, 1622, 998, ¹H-NMR (C₆D₆) δ : 0.72 (6H, d, *J*=6 Hz), 0.82 (3H, t, *J*=6 Hz), 1.2~1.6 (7H, br.), ca. 1.9 (2H, m), 3.04 (2H, t, *J*=6 Hz, changed to a doublet on addition of D₂O), 5.51 (1H, d, *J*=15 Hz), 5.74 (1H, dt, *J*=14 and 5 Hz), 6.10 (1H, dd, *J*=14 and 10 Hz) and 7.53 (1H, dd, *J*=15 and 10 Hz). The data were identical with those of (2*E*, 4*E*)-*N*-isobutyl-2, 4-decadienamamide, derived from (2*E*, 4*E*)-2, 4-decadienoic acid, which was synthesized from (*E*)-2-octenal by use of a Wittig reagent, triphenylphosphinecarboethoxymethylene.⁶⁾

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