

Acaricidal Daphnane Diterpenoids from *Trigonostemon reidioides* (KURZ) CRAIB roots

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A new daphnane diterpenoid, rediocide F (**1**), was isolated together with three known compounds, redioides A (**2**), C (**3**) and E (**4**), from the hexane extract of *Trigonostemon reidioides* roots by bioassay-guided fractionation for acaricidal activity on *Dermatophagoides pteronyssinus*, Thai common house dust mite. The structure of rediocide F (**1**) was established as the demethyl analog of rediocide C (**3**) on the basis of spectral analysis. Compounds **1**–**4** exhibited potent activity against *D. pteronyssinus* with respective LC₅₀ values of 2.53, 0.78, 5.59 and 0.92 μg/cm².

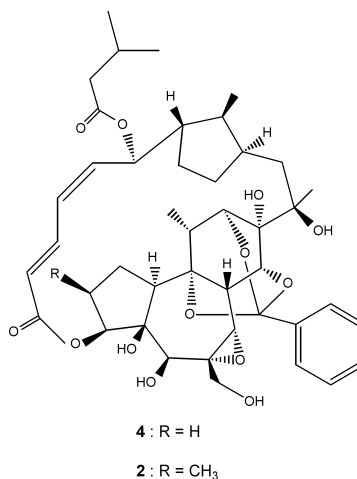
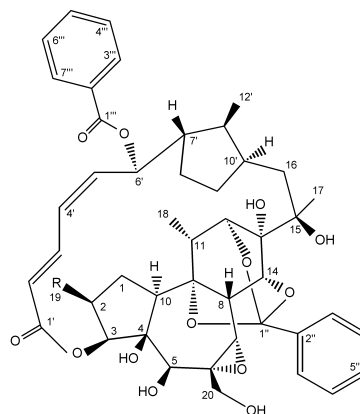
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House dust mite (HDM) is among the most important causative agent in the etiology of allergy, which is now considered to be a world wide problem.¹⁾ Present method to control HDM is to kill the mites or reducing HDM allergen.^{2–4)} Several synthetic acaricides, such as benzyl benzoate,⁵⁾ phenyl salicylate and pyrethroids, have been used for controlling HDM; however, only a few studies concentrate on the search for new chemical class of acaricides from natural sources.^{6–10)} In our search for novel acaricidal compounds from Thai medicinal plants, we came across the potent activity of the roots of *Trigonostemon reidioides* (KURZ) CRAIB (Euphorbiaceae; Thai name, Lot-tha-nong) whose extracts have been used in traditional medicine as antidote, expectorant and laxative. Previous phytochemical studies on this plant include the isolation of trigonostemone (a phenanthrenone),¹¹⁾ lotthanongine (an alkaloid),¹²⁾ and redioides A–E (daphnane diterpenoids).^{13,14)}

The hexane extract of *T. reidioides* roots, the most acaricidal active crude extract, was chromatographed on a column of silica gel, followed by a combination of reversed phase LPLC and preparative HPLC to furnish a new rediocide analog, rediocide F (**1**), along with three known compounds, redioides A (**2**), C (**3**) and E (**4**). Spectral data for **2**, **3** and **4** were identical to literature data in all respects (¹H- and ¹³C-NMR, MS, IR, and UV).

Rediocide F (**1**) was obtained as white powder. The IR and UV spectra of **1** were very similar to those of rediocide C (**3**). The molecular formula of **1** was determined by HR-MS (ESI-TOF) and ¹³C-NMR as C₄₅H₅₂O₁₃, which revealed that it comprised one methyl group less than that of **3**. ¹H- and ¹³C-NMR spectra of rediocide F (**1**) were similar to those of **3** except for the absence of signals for the C-19 methyl and the C-2 methine in **1** and the additional one methylene (δ_C 29.10; δ_H 2.02, 1.38), which was assigned to C-2 by the COSY, HMQC and HMBC correlations with H-1, H-2, H-3 and H-10. The COSY spectrum revealed the correlation between H-1 (δ_H 2.48) and H-2 (δ_H 1.38), H-3 and H-2 (δ_H 1.38), H-10 and H-1 (δ_H 2.48). The HMBC correlation no-

tably from H-1 (δ_H 2.02) to C-3 and C-4, H-2 (δ_H 2.02) to C-4 and C-10, H-2 (δ_H 1.38) to C-3 and C-4, H-3 to C-1 and C-2, and that from H-10 to C-1, C-2 and C-4 (Fig. 1). In addition, proton and carbon chemical shifts of this moiety in **1**



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Table 1. NMR Data for Rediocide F (1) in CDCl₃ (500 MHz for ¹H, and 125 MHz for ¹³C)

Position	δ _C , mult.	δ _H , mult., J in Hz	Position	δ _C , mult.	δ _H , mult., J in Hz
1	29.37 (t)	2.48 (m), 2.02 (m)	4'	130.70 (d)	6.29 (dd, 11.4, 11.4)
2	29.10 (t)	2.02 (m), 1.38 (m)	5'	136.60 (d)	5.69 (dd, 10.5, 10.5)
3	79.71 (d)	5.09 (d, 3.9)	6'	73.96 (d)	5.51 (dd, 10.6, 10.2)
4	82.14 (s)		7'	51.48 (d)	2.06 (m)
5	73.21 (d)	3.91 (br s)	8'	27.33 (t)	1.95 (m), 1.29 (m)
6	60.36 (s)		9'	31.71 (t)	1.62 (m), 1.43 (m)
7	64.58 (d)	3.39 (br s)	10'	40.61 (d)	1.70 (m)
8	35.55 (d)	4.73 (s)	11'	37.55 (d)	1.89 (m)
9	78.11 (s)		12'	17.01 (q)	0.83 (d, 7.2)
10	48.77 (d)	2.76 (dd, 13.9, 5.9)	1''	108.83 (s)	
11	37.00 (d)	2.97 (q, 6.7)	2''	138.81 (s)	
12	84.53 (d)	3.91 (br s)	3'', 7''	125.20 (d)	7.72 (dd, 7.2, 1.0)
13	71.70 (s)		4'', 6''	128.18 (d)	7.37 (m)
14	80.80 (d)	4.21 (s)	5''	129.36 (d)	7.37 (m)
15	76.17 (s)		1'''	165.83 (s)	
16	36.43 (t)	2.33 (d, 15.7), 1.53 (m)	2'''	130.42 (s)	
17	28.35 (q)	1.38 (s)	3''', 7'''	129.66 (d)	8.03 (d, 7.9)
18	18.68 (q)	1.62 (d, 6.4)	4''', 6'''	128.39 (d)	7.44 (dd, 7.8, 7.6)
19	—		5'''	133.00 (d)	7.56 (t, 7.2)
20	64.10 (t)	3.83 (d, 12.6); 3.67 (d, 12.6)	4-OH		3.16 (s)
1'	168.90 (s)		5-OH		3.67 (s)
2'	124.84 (d)	5.95 (d, 15.0)	13-OH		3.78 (s)
3'	139.02 (d)	7.81 (dd, 14.6, 11.8)	15-OH		1.44 (s)

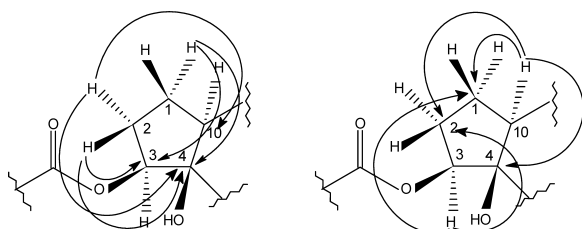


Fig. 1. Selected HMBC Correlations for Rediocide F (1)

were in comparable range to those of rediocide E (4), which is a 2-demethyl analog of rediocide A (2). On the basis of these spectral data, rediocide F (1) was identified as the 2-demethyl analog of rediocide C. All NOESY correlation data found for 1 were similar to those of known compounds 2–4, which suggested that the macrocyclic compound 1 possessed identical relative stereochemistry. Full assignments of protons and carbons for 1, established by combined analysis of COSY, HMQC, HMBC and NOESY spectra, are shown in Table 1.

Acaricidal activity on *D. pteronyssinus* of *T. reidioides* roots extracts and isolated compounds, 1–4, is shown in Table 2. The LC₅₀ values of each sample decreased in observation after 3, 5 and 7 d of incubation time, which indicated slow response when compared to the standard acaricide, benzyl benzoate. However, at the 7 d observation, the four isolated compounds, 1–4, showed significant killing effect against HDM with respective LC₅₀ values of 2.53, 0.78, 5.59 and 0.92 μg/cm², which are more potent than benzyl benzoate (LC₅₀ 6.6 μg/cm²).

This is the first report on the acaricidal activity of *T. reidioides* on house dust mite, and redioides were identified as active principles in this plant. Redioides A–E are known to exhibit insecticidal activities against mosquito larvae (*Aedes aegypti*) and ectoparasitic flea (*Ctenocephalides felis*).^{13,14} The potent acaricidal activity and structural uniqueness of

Table 2. Acaricidal Activity on *Dermatophagoides pteronyssinus* of *T. reidioides* Extracts and Redioides 1–4

Compound	LC ₅₀ (μg/cm ²)		
	3 d	5 d	7 d
Benzyl benzoate ^{a)}	7.2	6.9	6.6
Hexane extract	610	150	80
CH ₂ Cl ₂ extract	300	210	110
MeOH extract	>1140	>1140	>1140
Rediocide F (1)	14.94	2.19	0.92
Rediocide A (2)	9.49	6.10	2.53
Rediocide C (3)	5.40	1.37	0.78
Rediocide E (4)	16.55	5.04	5.59

a) Standard acaricidal compound.

the compounds deserve further investigation.

Experimental

General Procedures Melting point (mp) were determined on Electrothermal 9100 (uncorrected). Optical rotations were recorded on a JASCO DIP-370 polarimeter. IR and UV spectra were measured on SHIMADZU 440 FT-IR and MILTONROY 3000 ARRAY spectrophotometers, respectively. NMR spectra were obtained on BRUKER AV500D and DRX400 spectrometers. The chemical shifts are given in δ with TMS (tetramethylsilane) as an internal reference. Mass spectra were obtained on a Micromass LCT mass spectrometer. Merck silica gel no. 9385 (230–400 mesh) was used for column chromatography. LPLC was performed on a ISCO instrument. Preparative HPLC was performed on a WATERS 600 instrument (WATERS 996 photodiode array detector).

Plant Material Roots of *T. reidioides* were collected in October 2001 from Yasothon Province, Thailand. A voucher specimen (Wong 2348) is deposited at Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

Extraction and Isolation The dried, ground roots (2.8 kg) of *T. reidioides* were extracted in a soxhlet extractor by hexane, CH₂Cl₂ and MeOH, respectively. The hexane extract was found to be most active with a LC₅₀ value of 80 μg/cm² in the bioassay. The hexane extract (8.0 g) was chromatographed on a silica gel column, eluted with a gradient of hexane, CH₂Cl₂, EtOAc and MeOH, respectively, giving 9 fractions (A–I). The active I fraction (LC₅₀ 80 μg/cm²) was re-chromatographed under the same conditions, giving 5 fractions (11–15). The active I2 fraction (155 mg, LC₅₀

4.02 $\mu\text{g}/\text{cm}^2$) was subjected to LPLC (Lichroprep RP-18, 10 \times 240 mm; flow rate, 1 ml/min; detection, UV absorption at 254 nm) with the mobile phase H₂O/acetonitrile (5:95), giving a mixture of compounds **1**–**4** (66 mg), which was applied to separation by preparative HPLC (Nova-Pak HRC₁₈, 40 \times 100 mm; flow rate, 20 ml/min; detection, UV absorption at 254 nm) with the mobile phase H₂O/acetonitrile (30:70) yielded compounds **1** (14.9 mg, t_R 25.0 min), **2** (17.9 mg, t_R 28.5 min; $[\alpha]_D^{30} = -14^\circ$, $c=0.1$, CH₃OH), **3** (6.0 mg, t_R 30.0 min; $[\alpha]_D^{30} = -46^\circ$, $c=0.1$, MeOH) and **4** (10.1 mg, t_R 35.0 min; $[\alpha]_D^{30} = -124^\circ$, $c=0.1$, MeOH).

Rediocide F (**1**): White powder; mp 199–200 °C; $[\alpha]_D^{30} = -292.0^\circ$ ($c=0.1$, MeOH); UV (MeOH): $\lambda_{\text{max}}(\epsilon) = 236$ (19600), 256 (21200) nm; IR (KBr): $\nu_{\text{max}} = 3457, 2960, 1713, 1602, 1452, 1328, 1269 \text{ cm}^{-1}$; HR-MS (ESI-TOF): $m/z = 823.3318$ [M+Na]⁺ (Calcd for C₄₅H₅₂O₁₃Na, 823.3306); ¹H- and ¹³C-NMR: see Table 1.

Rediocide A (**2**): White powder; mp 193–195 °C; $[\alpha]_D^{30} = -124.0^\circ$ ($c=0.1$, MeOH); UV (MeOH): $\lambda_{\text{max}}(\epsilon) = 256$ (23000) nm; IR (KBr): $\nu_{\text{max}} = 3466, 2960, 1730, 1692, 1453, 1288, 1088, 1029 \text{ cm}^{-1}$; HR-MS (ESI-TOF): $m/z = 817.3793$ [M+Na]⁺ (Calcd for C₄₅H₅₂O₁₃Na, 817.3775).

Rediocide C (**3**): White powder; mp 191–193 °C; $[\alpha]_D^{30} = -46.0^\circ$ ($c=0.1$, MeOH); UV (MeOH): $\lambda_{\text{max}}(\epsilon) = 234$ (20600), 257 (20400) nm; IR (KBr): $\nu_{\text{max}} = 3420, 2960, 1717, 1603, 1453, 1268, 1107, 1029 \text{ cm}^{-1}$; HR-MS (ESI-TOF): $m/z = 837.3478$ [M+Na]⁺ (Calcd for C₄₅H₅₂O₁₃Na, 837.3462).

Rediocide E (**4**): White powder; mp 197–198 °C; $[\alpha]_D^{30} = -14.0^\circ$ ($c=0.1$, MeOH); UV (MeOH): $\lambda_{\text{max}}(\epsilon) = 256$ (15500) nm; IR (KBr): $\nu_{\text{max}} = 3451, 2958, 1729, 1692, 1452, 1335, 1288, 1119, 1026 \text{ cm}^{-1}$; HR-MS (ESI-TOF): $m/z = 803.3642$ [M+Na]⁺ (Calcd for C₄₅H₅₂O₁₃Na, 803.3619).

Bioassay The procedure of acaricidal activity was modified by Mahakitikun and *et al.*¹⁵ Acaricidal activity was tested on *D. pteronyssinus*, which have been continuously cultured at Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University. The mites were cultured in rat meal powder medium under controlled temperature (25 °C) and 75% relative humidity with saturated sodium chloride solution. Experiments were performed in the well of Siriraj Chamber (capacity 0.79 cm²/well). Ten mites were placed in each well, then applied 45 μl of the samples (in 20% acetone, 0.28–28 $\mu\text{g}/\text{cm}^2$ for the compounds) on the mites. Covered the well with glass plate of Siriraj Chamber and motility was observed using stereobionocular microscope after 3, 5 and 7 d incubation time. Mites that did not move when prodded with a fine brush were considered as dead, and percent deaths at each concentration were determined. Benzyl benzoate and 20% acetone in water were used as positive and negative control, respectively. All

experiments were replicated three times. The LC₅₀ values and 95% confident intervals were determined using Probit analysis method and calculated by Finney program.

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