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 $\mu\text{g}/\text{cm}^2$.

Key words *Trigonostemon reidioides*; acaricidal activity; rediocide; daphnane diterpenoid

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**

1 : R = H

3 : R = CH₃

4 : R = H

2 : R = CH₃

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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., <i>J</i> in Hz	Position	δ _C , mult.	δ _H , mult., <i>J</i> 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 rediociodes were identified as active principles in this plant. Rediociodes 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 Rediociodes **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 (II–V). The active II 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): λ_{max} (ϵ)=236 (19600), 256 (21200) nm; IR (KBr): ν_{max} =3457, 2960, 1713, 1602, 1452, 1328, 1269 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): λ_{max} (ϵ)=256 (23000) nm; IR (KBr): ν_{max} =3466, 2960, 1730, 1692, 1453, 1288, 1088, 1029 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): λ_{max} (ϵ)=234 (20600), 257 (20400) nm; IR (KBr): ν_{max} =3420, 2960, 1717, 1603, 1453, 1268, 1107, 1029 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): λ_{max} (ϵ)=256 (15500) nm; IR (KBr): ν_{max} =3451, 2958, 1729, 1692, 1452, 1335, 1288, 1119, 1026 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 Mahakit-tikun 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 cm^2 /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 stereo-bionocular 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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