Structure and Stereochemistry of Rediocide A, A Highly Modified Daphnane from *Trigonostemon reidioides* Exhibiting Potent Insecticidal Activity

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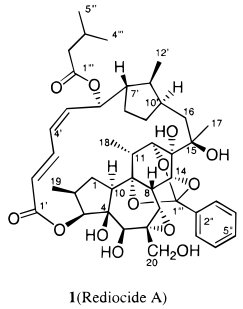
Parasites cause serious human and animal health hazards. Ivermectin,[1] a highly potent endectocide, and related compounds have been of tremendous value in treating endoparasites both in animals and in human river blindness,[1] a debilitating disease occurring in Africa, central and south America. When uncontrolled, ectoparasites can be a serious health threat to animals and in the case of companion animals (e.g., dogs and cats) can also create human health problems in the household. The emergence of resistance to a number of insecticides, and their general lack of effectiveness against ectoparasites such as fleas and ticks in companion animals, necessitates the discovery and development of environmentally friendly, novel insecticides. An orally active and potent ectoparasiticide would have substantial medical, economic and commercial benefits.

Screening of libraries of plant extracts against mosquito (*Aedes aegypti*) larvae,[2,3] followed by bioassay[2] guided fractionation of an active extract prepared from *Trigonostemon reidioides* Craib *(Euphorbiaceae*),[4] led to the discovery of rediocide A (**1**, Figure 1), a daphnane diterpenoid with an unusual 12-carbon polyketide extension at C-16 forming a macro-lactone at C-3, as a potent anti-flea insecticide. The isolation, structure elucidation, stereochemistry, computer modeling, and biological activity of rediocide A (**1**) are described here.

Gel permeation chromatography of a methylene chloride partition of the original methanolic extract of the roots of *T. redioides* on Sephadex LH-20 in methanol, followed by a combination of silica gel and reverse phase HPLC afforded 27 mg of rediocide A (yield 0.75% of the crude extract), which was crystallized from 2-propanol, mp. 213−15 °C, $[\alpha]^{22}_D$ +80° *(c,* 0.7, CH_3OH).

Structure Elucidation. FAB and ESI-MS analysis of rediocide A (**1**) produced pseudo-molecular ions at *m/z* 795 $(M + H)^+$ and 817 $(M + Na)^+$. FAB high-resolution mass measurement of the *m/z* 795 ion gave an exact mass value of *m/z* 795.3901 suggesting a molecular formula of $C_{44}H_{58}O_{13}$ (calcd 795.3955), which was corroborated by the ^{13}C NMR spectrum assuming five exchangeable protons. The IR spectrum showed absorption bands for hydroxyl (ν_{max} 3450 cm^{-1}), and ester/lactone (1730 and 1690 cm^{-1}) groups; the UV spectrum revealed an absorption band at λ_{max} 256 (ϵ = 20500) nm corresponding to the dienoate moiety.



1(Rediocide A)

Figure 1. Structure of rediocide A (**1**).

Analysis of the ^{13}C NMR, including a DEPT, spectrum of **1** revealed the presence of two-ester type carbonyls, a phenyl group, four olefinic methines, an ortho-ester carbon, five oxygen-bearing quaternary carbons, six oxy-methines, an oxy-methylene, eight aliphatic methines, five other methylenes, and six methyl groups. The proton-bearing carbons in the ^{13}C NMR spectrum were assigned by an HMQC experiment. The ^{13}C and ^1H NMR chemical shifts[5] are listed in Table 1. The $^1H-^1H$ spin systems of rediocide A were assigned by analysis of the $^1H-^1H$ COSY spectrum in 8 mM $CDCl_3$ solution which revealed six partial structures [(PS1) C10−C1−C2−C19−C3, (PS2) C11−C18, (PS3) C7−C8−C14, (PS4) C2′−C16, (PS5) C3′′−C7′′ and (PS6) C2′′′−C5′′′]. H-12 (J = 2.0 Hz) showed a weak correlation to H-14 due to a W-coupling, but showed no coupling with the vicinal proton H-11, due to a 90° dihedral angle (Dreiding model). A weak cross-peak was observed between H-11 and H-12 in the TOCSY spectrum. Likewise, H-8 showed weak cross-peaks with H-14 and H-7. The latter proton also showed a weak correlation with H-5 in the TOCSY spectrum. The TOCSY correlations supported all of the partial structures.

The connectivity of the partial structures (PS1−6) to each other, and to the remaining portion of the molecule, was accomplished by an HMBC experiment ($^nJ_{CH}$ = 7 Hz) and most of the two and three-bond HMBC correlations are summarized in Table 1. For example, the angular proton H-10 showed strong HMBC correlations to C-1, C-2, C-3, C-4, C-5, C-8, C-9, and C-11; H-5 to C-6 and C-7; H-7 to C-8, C-9, and C-14; H-14 to C-12 and H_3-18 to C-12. These correlations were critical both in connecting the partial structure PS1 to the rest of the molecule via a network of quaternary carbons and in establishing the highly oxygenated 5/7/6 ring system. The HMBC correlations of H_3-17 to C-13, C-15, and C-16 were helpful in connecting the tricyclic system to the large partial structure PS4. Similarly, HMBC correlations of H-2′ and H-3 to the shielded carbonyl (δ 168.83) confirmed the macro-lactone linkage of PS4 to C-3. HMBC correlations of H-2′′′ of PS6 and H-6′ to the remaining ester carbonyl C-1′′′ established the location of the isopentanoic ester group at C-6′. Finally, the benzoic acid ortho-ester connectivity to C-9, C-12, and C-14 was determined by the HMBC correlations of H-12, H-14, and the aromatic proton H-′′3 to the ortho-ester C-′′1 (δ 108.82). These data helped in establishing the planar structure of rediocide A (**1**, Figure 1).

Stereochemistry. The stereochemistry of rediocide A was elucidated by the measurement of *J* couplings, NOESY spectroscopy, and computer modeling. The geometry of the $\Delta^{2'}$ and $\Delta^{4'}$ olefins was established as *E* and *Z*, respectively, on the basis of

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(4) Stems, leaves and wood of *Trigonostemon reidioides* Craib *(Euphorbiaceae*) were collected in Rat Buri Province, Thailand, in December, 1988. Voucher specimens (Nanakorn 88213) are deposited in the herbarium of the New York Botanical Garden. The authors wish to thank the National Science Council of Thailand for support of, and collaboration in, this project.

(5) The ^1H shifts of H-5, H-10, and one of the H_2-20 signals experienced an upfield shift of ~0.1 ppm, and H-11 experienced a downfield shift of 0.1 ppm when the ^1H NMR spectrum was recorded at 40 mM concentrations.

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Table 1: ^{1}H and ^{13}C NMR Assignments of Rediocide A (8mM) in $CDCl_3$ Solutions[a]

position	δC[a]	δH[b], mult, *J* in Hz	HMBC[c] (C → H)	position	δC[a]	δH[b], mult, *J* in Hz	HMBC[c] (C → H)	position	δC[a]	δH[b], mult, *J* in Hz	HMBC[c] (C → H)
1	36.22	β: 2.2, m	H-3, 10, H_3-19	17	28.28	1.36, s	H-16β	1″	108.82	–	H-12, 14, 3″
		α 1.95, m		18	18.48	1.57, d, 7.6	H-11	2″	138.73	–	H-4″, 6″
2	35.45	1.70, m	H-10, H_3-19	19	13.34	1.10, d, 6.8		3″	125.14	7.68, dd, 8, 1.6	H-4″, 5″, 7″
3	82.17	4.88, d, 4.6	H-1α, 5, 10, H_3-19, OH-4	20	64.81	3.77, ABq, 12	H-5	4″	128.15	7.38, m	H-3″, 6″
4	82.56	–	H-1α,10, OH-4	1′	168.83	–	H-3, 2′, 3′	5″	129.32	7.35, m	H-4″, 3″
5	73.13	3.96, brs	H-3, 10, H_2-20, OH-4	2′	124.59	5.92, d, 14.9	H-4′	6″	128.15	7.38, m	H-7″, 4″
6	60.53	–	H-5, 7, 8, H_2-20, OH-5	3′	138.73	7.70, dd, 14.8, 11.2	H-4′, 5′	7″	125.14	7.68, dd, 8, 1.6	H-6″, 3″
7	64.88	3.43, brs	H-5, 8, 20	4′	130.27	6.23, dd, 11.2, 11.2	H-2′	1‴	172.21	–	H-6′, 2‴
8	35.67	4.66, d,[e] 1.2	H-7, 10	5′	136.86	5.57, dd, 10.8, 10.8		2‴	43.80	2.16, d, 7.0	H-3‴, H_3-4‴, 5‴
9	78.04	–	H-7, 8, 10	6′	72.77	5.31, dd, 10.4, 10.4	H-4′, 8′β, 11′	3‴	25.94	2.09, m	H-2‴, H_3-4‴, 5‴
10	48.43	2.90, dd, 13.6, 6.0	H-3, 18, OH-4	7′	51.59	1.90, m	H-5′, 6′, 8′β, H_3-12′	4‴	22.36	0.93, d, 6.4	H-2‴, H_3-5‴
11	36.99	2.83, q, 6.5	H-10, H_3-18	8′	27.23	β: 1.85, m	H-11′	5‴	22.45	0.93, d, 6.4	H-2‴, H_3-4‴
12	84.36	3.90, brd, 2.0	H-14, H_3-18			α 1.16, m		4-OH		2.79, s	
13	71.69	–	H-16α, 16β, H_3-17, OH-15	9′	31.72	β: 1.42, m	H-16α, 11′	5-OH		3.39, s	
14	80.61	4.20, dd,[d] 1.2, 2.0	H-7, 8			α 1.63, m		13-OH		3.79, s	
15	76.49	–	H-12, 16α, 16β, H_3-17, OH-15	10′	40.59	1.65, m	H-8′β, 11′, 12′, 16α	15-OH		1.50, s	
16	36.44	β: 1.50, m	H_3-17	11′	37.82	1.75. m	H-16α, 8′β, H_3-12′				
		α: 2.34, d, 16		12′	16.90	0.80, d, 7.2	H-11′				

[a] Recorded at 100 MHz. [b] Recorded at 400 MHz. [c] Recorded at 500 MHz, HMBC ($^nJ_{CH}$ = 7 Hz). [d] Resolution enhanced.

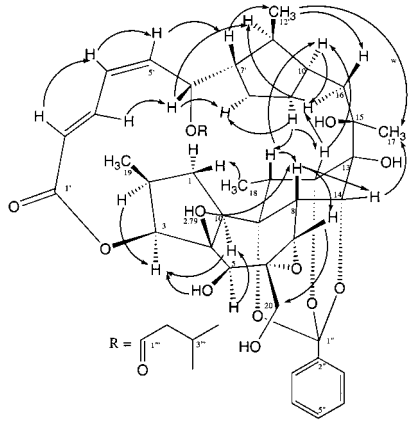

Figure 2. Conformation of rediocide A (**1**) with selected NOESY correlations (mix time = 300 ms).

the large ($J_{H2',H3'}$ = 14.8 Hz) and small ($J_{H4',H5'}$ = 10.8 Hz) coupling constants between the olefinic protons and confirmed by NOESY correlations. The 400 MHz NOESY spectrum (Figure 2) of rediocide A showed strong correlations of H-5 to H-3 and H-10; H-3 to H-2, and H-10 to H-1α (δ 1.95) thus orienting these protons on the α-face of the molecule, assuming the daphnane absolute stereochemistry. A 1,3-diaxial relationship between H-8 and H-11 and their β-orientation was established on the basis of strong NOESY correlations of H-8 to H-11 and OH-4. H-8 also showed correlations to H-7 and H-14, thus placing H-14 in the equatorial position. The axial H-11 exhibited correlations to H_3-18 and H-12 indicating that H-12 is equatorial. This is consistent with the minimal coupling ($J_{H11,H12}$ = <1 Hz) between H-11 and H-12. These and other NOESY correlations indicated *trans-trans-trans* tricyclic ring fusion and a boat conformation for the seven-membered ring, as in other daphnanes. On the other hand, a chair conformation is proposed for the six-membered ring, in contrast to other ortho-ester-containing daphnanes.[6] The 1,3,5-triaxially connected ortho-ester group at C-9, C-12, and C-14 is oriented at the α-face of the molecule.

The C-17 methyl group exhibited a strong NOESY cross-peak with H-14 and a very weak cross-peak with H_3-12′; the OH-13 gave similar correlations to H-12 and H-14, and H-16α (δ 2.34) showed a correlation to H-12, suggesting an axial orientation of C-15 at C-13. These NOEs are possible only if the C-15 hydroxy group is positioned on the β-face of the cyclohexane ring. This was further supported by the NOESY correlations of OH-15 to H-8.

The strong NOESY correlations of H_3-12′ to H-7′, and H-16β (δ 1.51) as well as correlations of H-6′ to H-3′, H-8′α (δ 1.16), and H-11′ (δ 1.70) and those of H-10′ with H-11, H-12, and H-16α (δ 2.34) established the stereochemistry at C6′, C-7′, C-10′, and C-11′ as shown. H-11 and H-12 also showed NOESY correlations with H-9′α, indicating that the macrocyclic ring swings back and forth on the top face of the cyclohexyl ring. A Dreiding model was used to confirm the spatial interatomic distances, and these were verified with computer modeling.[7] The distances between the protons showing NOESY cross-peaks in the unconstrained minimized structure were in the range of 2.24−3.08 Å except for the H-3−OH-5 (3.80 Å), H-14−OH-15 (3.13 Å), H-12−OH-15 (3.46 Å), and H_3-17−H_3-12′ (4.44 Å). On the basis of all of the spectral data, structure **1** (Figure 1) is assigned to rediocide A.

Biological Activity. Rediocide A (**1**) showed potent activities against mosquito larvae in an in vitro assay[2,3] and against fleas (*Ctenocephalides felis*) in an artificial membrane feeding system and exhibited LD_{90} values of 1 and 0.25 ppm, respectively. In view of the structural similarity of rediocide A and the phorbol and phorbol esters (phorbol, 12-acetate, 13,20-diacetate, 12,13-diacetate, 13-butyrate, and 12-decanoate), were tested in the latter assay. None of the compounds showed any activity at 10 ppm. Rediocide A (**1**) is one of the most potent anti-flea compounds discovered to-date in our research program (e.g., ivermectin exhibited LD_{90} value of 10 ppm against fleas).

Rediocide A (**1**) has three unique features when compared with daphnane and tigliane diterpenoids.[6] Like kraussiamin[8] and wikstroelides,[6b] it is derived from a mixed terpenoid and polyketide biosynthesis, but unlike kraussiamin and wikstroelides, rediocide A consists of an unprecedented 12-carbon polyketide extension at C-16; it has an 9,12,14-ortho-ester group instead of the more common 9,13,14-ortho-ester, resulting in a chair conformation for the cyclohexyl ring; and it is a highly potent insecticide. Trigonostemone, a phenanthrenone, is the only other compound reported[9] from the genus *Trigonostemon*.

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(7) A 3D structure of rediocide A was generated by using the stereochemistry from the ChemDraw rendering. This structure was energy minimized with MMFF94S force field (Halgren, T. A. *J. Comput. Chem.* **1994**, *20*, 720) a dielectric constant of 78, a nonbonded cutoff of 7 Å, convergence criterion for gradient <0.001, and truncated Newton optimization algorithm. This structure was used to project 40 structures with the aid of a distance geometry program using nOe constraints. These 40 structures were then energy-minimized without any constraints using the same conditions as above. The individual average interproton distances measured in these 40 conformers were found to be within the range of the nOe constraints.

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