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Coupling of *ent*-Cyclic Peroxide and Ircinol A, Two Biologically Active Natural Marine Products

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An acidic *ent*-cyclic peroxide was isolated from a sponge, *Plakotis* sp., and showed activity against leishmaniasis and pathogenic fungi. To improve the activity of this compound, we coupled the acidic *ent*-cyclic at the C1 position of ircinol A. Compound 3 exhibited significant activity against *Leishmania mexicana* and fungi with IC_{50} values of 0.7 and 0.3-34 μg/mL, respectively. The yield of compound 3 was 98%.

Key words: *ent*-cyclic peroxide, Ircinol A, Leishmania, Coupling

Introduction

Many cyclic peroxides have been isolated from marine organisms (Gunasekera et al., 1990). These compounds exhibit antimicrobial activity (Lim et al., 2005), ichthyotoxicity and cytotoxicity (Perry et al., 2001). Many peroxides have shown strong *in vitro* antiproliferative effects against promastigotes of *Leishmania mexicana*, a flagellate protozoan that causes leishmaniasis. Leishmaniasis is a tropical disease that affects an estimated 350 million people in equatorial Asia, Africa and Central and South America. This disease infects 1.5 to 2 million people annually in many of the world's poorest countries (WHO, 2000). In Central and South America, the causative agent is the protozoan *Leishmanania mexicana*, which is maintained in the rodent population and transmitted by flies. Pentostam and glycantil, the most common drugs for treating visceral leishmaniasis, contain pentavalent antimonials that have cardiotoxic effects at the recommended doses. Amphotericin B and azoles, which are alternative drugs, have equally unpleasant side effects. The need to discover alternative treatments has led to our program to couple various different bioactive natural products for potential use in the therapy of leishmaniasis (Compagnone et al., 1998).

Peroxide-containing marine natural products have shown activity against *Toxoplasma gondii*, an opportunistic parasitic infection in AIDS, as well as the malarial parasite *Plasmodium falciparum* (Longley et al., 1993; El Sayed et al., 2001). The cyclic peroxide (compound 1) discussed here affects the proliferation of *L. mexicana* promastigotes (Compagnone et al., 1998).

Ircinol A was first isolated from an Okinawan marine sponge, *Amphimedon* sp., in 1994 (Masashi et al., 1994). Ircinol A is a manzamine-type alkaloid and consists of a complex heterocyclic ring system attached to a β-carboline moiety. Ircinol A (compound 2) is cytotoxic against L1210 cells (IC_{50}: 2.4 μg/mL) and KB cells (IC_{50}: 6.1 μg/mL) and inhibits endothelin-converting enzyme (IC_{50}: 55 μg/mL). Ircinol A is active against *Mycobacterium tuberculosis* (H37-Rv), with a minimum inhibitory concentration (MIC) at 1.93 μg/mL, against *Plasmodium falciparum* (D6 clone) *in vitro* (IC_{50}: 2,400 ng/mL), and against *P. falciparum* (chlorine-resistant W2 clone) *in vitro* (IC_{50}: 3,100 ng/mL) (Longley et al., 1993; Ang et al., 2000; El Sayed et al., 2001).

In order to enhance its antileishmanial and other biological activities, we examined the chemical coupling of and acidic *ent*-cyclic peroxide and ircinol A and evaluated the activity of the product against organisms causing infectious diseases.

Materials and Methods

Reaction procedure

We dissolved 50 mg of ircinol A (0.075 M) in methylene chloride (0.5 mL). To this solution, the acidic cyclic peroxide (0.075 M, 50 mg) and dimethyl aminopyridine (catalytic amount) were added and

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stirred for about 5 minutes. This was followed by the addition of N,N'-dicyclohexylcarbodiimide (0.075 M, 50 mg). The reaction was stirred at room temperature and its progress was monitored using thin layer chromatography (TLC). The reaction was stopped after 18 hours. The reaction mixture was filtered and evaporated. The residue was fractionated on silica gel G254 2,000 μM, using acetone-hexane, (80:20). This afforded compound 3 (98 mg) as the major compound in the form of a yellowish gum (Fig. 1).

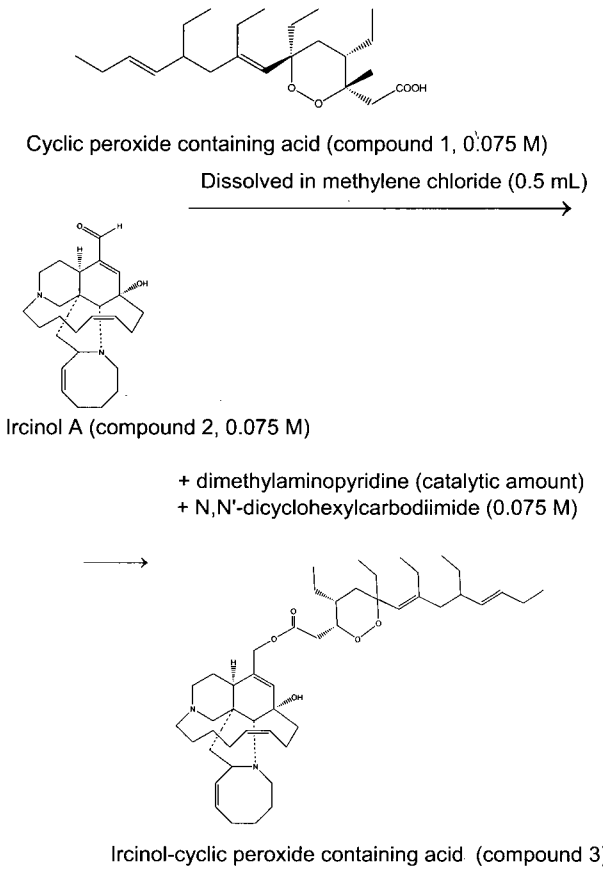

Fig. 1. Semi-synthetic scheme for the preparation of the ircinol-cyclic peroxide ester

General experimental procedures

Melting points are uncorrected. One- (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DRX-400 spectrometer. The chemical shift, with δ values expressed in parts per million (ppm), are relative to the residual solvent signals with resonances at δ_H/δ_C 7.26/77.0 ($CDCl_3$). Electrospray ionization-Fourier transform mass spectrometery (ESI-FTMS) was performed on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution high-performance liquid chromatography (HPLC)-FT spectrometer by direct injection into an electrospray interface. TLC was performed on aluminum sheets (Silica gel 60 F_{254}, Merck KGaA, Germany) with an acetone:hexane (80:20) developing system.

Antimicrobial and antileishmanial activity test

Antimicrobial activity was tested by using Holla et al. (2005) and the bioassay used parasites of the trypanosom family characterized as *Leishmania mexicana* (NR strain). The cells were cultured in liver infusion tryptose (LIT) medium, supplemented with 10% fetal calf serum (GIBCO) and transferred to fresh medium every 5 days. The bioassays were carried out in triplicate. Different concentrations of the drugs dissolved in demethyl sulfoxide were added to a suspension of cells (2×10^6) in 10 mL of phosphate-buffered saline (PBS) at pH 7.2. DMSO alone was used as a control. The cultures were placed in a New Bauer chamber and monitored daily for 96 hours using light dispersion at 560 nm to determine cell density. The cells were also monitored using a Nikon-Diaphot microscope to determine the parasite mobi-lity and membrane integrity (Compagnone et al., 1998).

Results and Discussion

The acidic *ent*-cyclic peroxide (compound 1, Lim et al, 2006) was obtained from an ethanol extract of a sponge, *Plakortis* sp. (1.3 kg, wet weight), after repeated chromatography on silica gel, gel permeation and reverse-phase (RP)-HPLC. This compound was a colorless oil with an $[\alpha]_D^{25}$ of -165 (*c* 0.18, $CHCl_3$). The HR-ESI-MS spectrum showed a molecular ion peak (M+1) at *m/z* 367.2770 (calc. for $C_{22}H_{39}O_4$, 367.2961) and three degrees of unsaturation.

Ircinol cyclic peroxide (compound 3) was obtained as a yellowish gum and its molecular formula was determined to be $C_{47}H_{74}N_2O_5$ using positive ion HR-ESI-FTMS $[M+H]^+$ 747.4382 (Calc. 747.4228). The proton, carbon, distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple quantum coherence (HMQC) NMR experiments allowed the assignment of 14 methane, 22 methylene, and 5 methyl groups and 7 quaternary centers (Tables 1, 2). In the heteronuclear multiple bond correlation (HMBC) spectrum, no 4*J* correlations were observed between H1 (4.83, 4.92) and H2' (2.39, 2.98), but we assumed that the acidic *ent*-peroxide was coupled at the C1 position of ircinol A. Since a carboxyl acid contains the COOH group, and the hydrogen in the

Table 1. ^{1}H NMR data for the ircinol-cyclic peroxide ($CDCl_3$)

No.	^{1}H	No.	^{1}H
1	4.92 (d, 12.3)	34	4.03 (br s)
	4.83 (d, 12.3)		
10	-	35	1.92 (m)
			1.57 (m)
11	5.85 (s)	36	2.66 (d, 11.5)
			2.14 (d, 11.5)
12	-	1'	-
13	1.73 (m)	2'	2.98 (dd, 15.7, 9.4)
	1.35 (m)		2.39 (d, 15.9)
14	2.09 (m)	3'	4.40 (br t, 4.50)
	1.87 (m)		
15	5.11 (br s)	4'	1.91 (m)
16	4.97 (br s)	5'	1.65 (dd, 12.9, 2.6)
			1.22 (m)
17	1.45 (m)	6'	7.74 (br s)
	1.61 (m)		
18	1.69 (m)	7'	5.09 (s)
	1.88 (m)		
19	1.65 (m)	8'	
	2.15 (m)		
20	2.33 (m)	9'	1.93 (dd, 14.0, 7.5)
	2.65 (m)		
21	-	10'	1.19 (dd, 15.1, 7.8)
22	2.96 (m)	11'	5.07 (dd, 15.3, 7.3)
	2.87 (m)		
23	1.96 (m)	12'	5.32 (dt, 6.1)
	1.44 (m)		
24	1.90 (m)	13'	1.93 (m)
			1.91 (m)
25	-	14'	0.93 (3H, m)
26	3.22 (s)	15'	1.22 (m)
			1.12 (m)
27	-	16'	0.88 (3H, m)
28	2.69 (m)	17'	1.52 (2H, m)
	1.73 (m)		
29	2.13 (m)	18'	0.83 (3H, m)
	1.63 (m)		
30	2.13 (m)	19'	2.25 (m)
	1.63 (m)		2.08 (m)
31	2.10 (m)	20'	0.93 (3H, m)
	1.67 (m)		
32	5.80 (br s)	21'	1.38 (m)
			1.14 (m)
33	5.39 (br s)	22'	0.80 (3H, m)

Table 2. ^{13}C NMR data for the ircinol-cyclic peroxide ($CDCl_3$)

No.	^{13}C	No.	^{13}C
1	68.8 (t)	34	55.0 (d)
10	142.6 (s)	35	44.7 (t)
11	135.8 (d)	36	67.1 (t)
12	69.5 (s)	1'	177.1 (s)
13	40.4 (t)	2'	35.5 (t)
14	21.8 (t)	3'	78.3 (s)
15	129.1 (d)	4'	142.0 (s)
16	132.1 (d)	5'	126.6 (d)
17	27.1 (t)	6'	84.0 (s)
18	28.4 (t)	7'	42.3 (d)
19	26.2 (t)	8'	35.1 (d)
20	53.8 (t)	9'	27.6 (t)
21	-	10'	42.3 (d)
22	51.1 (t)	11'	132.9 (d)
23	32.0 (t)	12'	131.4 (d)
24	39.0 (d)	13'	25.4 (t)
25	47.1 (s)	14'	13.7 (q)
26	75.0 (d)	15'	24.7 (t)
27	-	16'	11.8 (q)
28	49.8 (t)	17'	31.1 (t)
29	31.9 (t)	18'	7.4 (q)
30	26.0 (t)	19'	22.5 (t)
31	30.0 (t)	20'	10.7 (q)
32	134.8 (d)	21'	32.6 (t)
33	130.1 (d)	22'	11.7 (q)

group is linked to an alcohol group in some reactions, compound 1 would be esterified on coupling with the alcohol group of ircinol A in the presence of the dimethyl aminopyridine catalyst.

The effects of compound 1 on the proliferation of *L.mexicana* promastigotes and its activity against *Candida albicans*, *Cryptococcus neoformans,* and *Aspergillus funigatus* have already been checked (Lim et al., 2005). Compound 1 was very potent against the protozoan (IC_{50} 1.0 μg/mL) and exhibited significant antifungal activity *in vitro* with IC_{50} values of 0.5-50 μg/mL. Compound 3 had higher against the protozoan of *L. mexicana* promastigotes and fungi of *C. neoformans* and *A. funigatus* (0.7 and 0.3-34 μg/mL, respectively), whereas it was less effecttive against *C. albicans* (0.8 μg/mL) than compound 1 (Table 3).

Table 3. Biological activities of compounds 1 and 3 against *Leishmania mexicana* and fungi. The IC_{50} values are expressed as μg/mL

Compound	*Leishmania mexicana*	Microbes (μg/mL)		
		Candida albicans	*Cryptococcus neoformans*	*Aspergillus funigatus*
Compound 1	1.0	0.6	0.5	50
Compound 3	0.7	0.8	0.3	34

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