Pergamon 0031-9422(95)00265-O

Phytochemistry, Vol. 40, No. 1, pp. 317–320, 1995

0031-9422/95 $9.50 + 0.00

4-QUINOLINONE ALKALOIDS FROM *DICTYOLOMA PERUVIANA*

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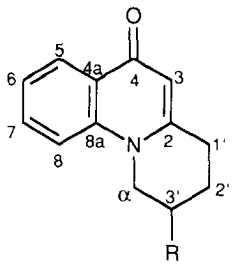
(*Received in revised form* 27 *February* 1995)

Key Word Index—*Dictyoloma peruviana*; Rutaceae; stem-bark; 4-quinolinone alkaloids; antileishmanial activity.

Abstract—The stem-bark of *Dictyoloma peruviana* yielded two new piperidino [1,2-a] 4-quinolinones, dictyolomide A and dictyolomide B. Their structures were established by NMR spectroscopy.

INTRODUCTION

Only two species belong to the genus *Dictyoloma*: *D. incanescens* (syn. *D. vandellianum*) and *D. peruviana* and they are widespread in Bolivia. *Dictyoloma incanescens* is known to contain indole alkaloids [1, 2], 2-quinolinone alkaloids [1], prenylated chromones [3] and limonoids [1]. *Dictyoloma peruviana* is a small tree used in folk medicine for the treatment of leishmaniasis; to the best of our knowledge, it has not been the subject of phytochemical investigations. As part of a cooperative programme on the search for new antileishmaniasis agents from natural sources, bioassay-guided separations were conducted which led to the isolation and structural elucidation of two 4-quinolinone alkaloids from the stem bark of this species.



1 R = $-CH_2-CH_2-CH=CH-CH_2-CH_3$ (*cis*; 4' 5' 6' 7' 8' 9')

2 R = $-CHOH-CH_2-CH_2-CH_2-CH_2-CH_3$ (4' 5' 6' 7' 8' 9')

RESULTS AND DISCUSSION

Dictyoloma peruviana was collected in the Carrasco province of Bolivia. *In vitro* antileishmanial activity tests were performed on various strains of promastigote forms of *Leishmania* species. Crude extracts and pure compounds, aseptically dissolved in liquid medium and DMSO (final concentration of DMSO less than 0.1%), were assayed in triplicate as previously described [4]. Biological activity was concentrated in the ethyl acetate fraction, which contained two compounds (named dictyolomides A and B), for which structures **1** and **2** are proposed. Antileishmanial activity could be attributed to these compounds which induced complete lysis of parasites at 100 $\mu g\,ml^{-1}$ (Table 1).

The two compounds belonged to the same class of molecules and mass spectrometry allowed determination of M_r of 281 ($C_{19}H_{23}NO$) for **1** and 299 ($C_{19}H_{25}NO_2$) for **2**. The 4-quinolone skeleton [5] was recognized on the basis of UV spectra and ^1H NMR signals at 6.30 ppm (H-3) and 8.47 ppm (H-5 deshielded by a *peri*-carbonyl group). The chemical shifts of C-4 carbonyls at 176.8 ppm in **1** and 176.7 ppm in **2** are characteristic of a vinylogous amide.

On the basis of the hypothesis of **1** being a 4-quinolone, its elemental composition indicates the presence of two supplementary unsaturations. Their location and the proposed structure **1** is based on the assignment of ^1H and ^{13}C NMR spectra with the help of 2-D correlation experiments. These unsaturations are located in the $C_{10}H_{19}$ fragment anchored at positions 1 and 2 of the quinolone. The ^1H NMR spectrum of **1** displayed five signals, corresponding to the protons of rings A and B of the quinolone. HMQC and HMBC experiments allowed identification of the carbon signals for these rings. Proton signals not included in the quinolone system were observed at δ5.45 and 5.35 (ethylenic protons attached to

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Table 1. *In vitro* activity of *D. peruviana* extracts and of alkaloids **1** and **2** on promastigote forms of *Leishmania* species

Extract	Strain*	Concentration ($\mu g\,ml^{-1}$)†			
		100	50	25	10
Petrol	*L. a.*	+	0	0	—
	L. b.	+ +	+ +	0	—
Ethyl acetate	*L. a.*	+ + +	+ +	0	—
	L. b.	+ + +	+	0	—
Alcoholic	*L. a.*	+ +	+	0	—
	L. b.	+	+	0	—
Alkaloid mixture	*L. a.*	+ + +	+ + +	+ +	—
	L. b.	+ +	+ +	0	—
Alkaloid **1**	*L. a.*	+ + +	+ + +	+ +	—
	L. b.	+ +	+	0	—
Alkaloid **2**	*L. a.*	+ + +	+	0	—
	L. b.	+ +	+	+	—
Pentamidine	*L. a.*	—	—	—	+ + +
	L. b.	—	—	—	+ + +

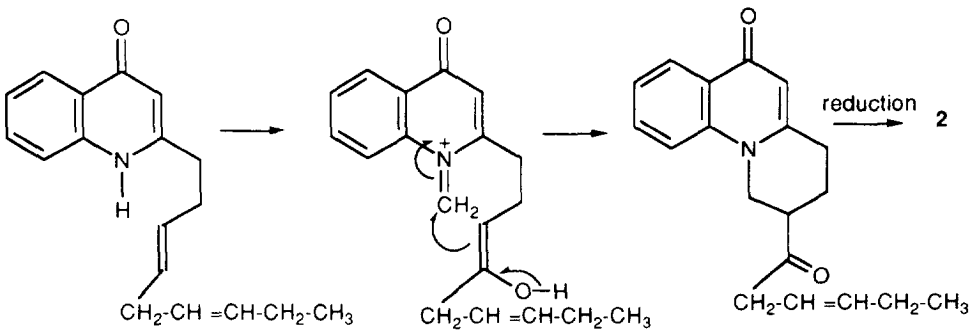
* *L. a.* = *Leishmania amazonensis*; *L. b.* = *Leishmania braziliensis*.

†0: Promastigotes identical to control; + : 75% promastigotes, with a few degenerative forms; + +: 50% promastigotes, with a few degenerative forms; + + +: no promastigotes, total lysis of parasites.

Table 2. ^{13}C NMR data and HMBC correlations of alkaloids **1** and **2**

C	**1**	**2**	HMBC correlations	
			1	**2**
2	152.1	152.7	H-3, H-α, H-1′	H-3, H-α, H-1′, H-2′
3	109.7	109.0	H-1′	H-1′
4	176.7	176.8	H-5	H-5
4a	126.5	126.3	H-3, H-6,	H-3, H-6,
5	126.8	126.3	H-7, H-8	H-7, H-8
6	124.0	123.8	H-7, H-8	H-8
7	132.0	131.9	H-5, H-6, H-8	H-5
8	114.8	115.2	H-6	H-6
8a	141.3	141.3	H-5, H-7, Hα	H-5, H-7, Hα
α	51.2	47.2	H-4′	H-4′
1′	30.0	29.9	H-3	H-3, H-2′
2′	25.0	22.2	H-α, H-1′, H-4′	H-α, H-1′
3′	33.8	40.0	H-α, H-1′, H-5′	H-α, H-1′, H-2′
4′	34.0	73.4	H-6′, H-7′	H-α
5′	24.1	34.7	H-4′, H-6′, H-7′	
6′	127.8	25.2	H-4′, H-5′, H-8′	H-4′
7′	133.0	31.8	H-5′, H-8′, H-9′	H-8′, H-9′
8′	20.6	22.6	H-6′, H-7′, H-9′	H-7′, H-9′
9′	14.5	14.0	H-8′	H-8′

carbons at δ127.8 and 133.0), at δ4.30 (*dd*, J = 12.5 and 5 Hz) and 3.59 (*dd*, J = 12.5 and 10.5 Hz) as the AB part of an ABX system, between δ3.0 and 1.45 (11H, multiplets) and at δ1.00 (*t*, J = 7 Hz) for one terminal methyl. The ^{13}C NMR spectrum showed 19 carbons, 11 downfield of chloroform, with the nine sp^2 carbons of the 4-quinolinone part and the two supplementary ethylenic methines at δ127.8 and 133.0 and, at higher field, one methyl, one methine sp^3 and six methylenes (Table 2). The ABX system was assigned to a N–CH_2–CH– moiety with attachment of the nitrogen based on the chemical shift of the methylene (δ51.2). Two adjacent methylenes were detected in the COSY experiment at δ3.0 ppm (*m*, 2H), 2.05 (*m*, 1H) and 1.45 (*m*, 1H). Coupling of the latter



Scheme 1. Proposed rate for biogenesis of alkaloids **1** and **2**.

methylene with the aliphatic CH could not be determined because of overlap but the other methylene was coupled at long-range with H-3 of the quinolone. This CH_2–CH_2 fragment is probably attached to C-2 of the quinolone. The HMBC experiment allowed linkage of these fragments through observation of long-range couplings between the N–CH_2 and quaternary carbon atoms of the quinolone (C-8a at δ141.3 and C-2 at δ152.1) and between H-1′ of the CH_2–CH_2 fragment and C-2. The number of unsaturations and the presence of one double bond imply one ring and one chain for the non-quinolone fragment; the chain must therefore be attached to the aliphatic methine. The composition of the chain was determined as C_6H_{11} from the base peak in the mass spectrum (*m/z* 198); the count of methylenes (three) indicates that the chain is linear and contains a disubstituted double bond. Identification of the chain requires the presence of a supplementary ring as a piperidine substituted at C-3′ (alkaloid numbering) by the alkenyl chain. Final location of the unsaturation in the chain is based on observation of a long-range correlation between the protons of the methyl (triplet at δ1.0, J = 7 Hz and, therefore, CH_3–CH_2–) and an ethylenic carbon at δ133.0. Compound **1**, dictyolomide A, is therefore a piperidino [1,2-a] 4-quinolinone substituted by a 3-hexenyl chain at C-3′. The *cis*-configuration of the double bond is deduced from the chemical shift of the terminal methylene (δ20.6) shielded as in the *cis*-hex-3-ene at δ20.7.

The second compound (**2**) showed a $[M]^+$ at *m/z* 299, which corresponds to a molecular formula of $C_{19}H_{25}NO_2$. This suggested that **2** is a hydroxyl derivative of **1**. The ^1H and ^{13}C NMR spectra of **2** displayed many similarities with those of **1** (Table 2). The major differences were the absence of the ethylenic system in the linear chain and the presence of a hydroxymethine group at δ_H 3.60 and δ_C 73.4 in **2**. This proton shows coupling with H-3′ of the piperido ring in the COSY spectrum and a cross-peak with C-α in the HMBC experiment; the reverse correlation between one of the H-α protons (δ3.96) and this hydroxymethine carbon is also observed. Thus, the chain is hydroxylated at position 4′ and the appendage fragment corresponds to a hexan-1-ol in alkaloid **2**.

The isolation in two other species of Rutaceae of 4-quinolinone alkaloids with an alkyl chain at C-2, 2-(3′-6′-nonadienyl)-4-quinolone [6] and 1-methyl-2-nonyl-4-quinolone [7], allows us to propose a biogenetic scheme for compounds **1** and **2** involving a Mannich reaction for the formation of the third ring (Scheme 1). This reaction would leave a carbonyl function at position 4′, which is further functionalized in compound **2**.

EXPERIMENTAL

UV spectra measured in MeOH. ^1H and ^{13}C NMR were recorded at 300 and 75 MHz respectively, in $CDCl_3$. 2-D exps were performed using standard Bruker microprograms. Hardware modifications of the spectrometer allowed acquisition of C–H correlations in the reverse mode (HMQC and HMBC (d_4 = 70 msec)).

Plant material. Dictyoloma peruviana Planchon was collected at 'Valle del Sacta', Cochabamba area, Carrasco province (Bolivia), in November 1991. A voucher specimen is deposited at the Herbarium of San Andrès University in La Paz.

Extraction and purification of alkaloids. Ground stem bark (1097 g) was wetted with 50% NH_4OH and extracted (× 7) with $CHCl_3$. The extracts were concd (200 ml) under red. pres. then extracted with 5% HCl. The aq. phase was made alkaline with 50% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried (Na_2SO_4) and then evapd *in vacuo* to give 1.38 g crude alkaloid mixt. (1.25 g kg^{-1}). The $CHCl_3$ residue was chromatographed on a column of silica gel (48 g) eluting with increasing percentages of $CHCl_3$–MeOH: 49:1, 97.3, 19:1 and 9:1. From the $CHCl_3$–MeOH (97:3) eluate, alkaloid **1** was obtained in frs 75–86; the $CHCl_3$–MeOH (19:1) eluate, fr. 135–150, yielded alkaloid **2**.

Dictyolomide A (**1**). $[\alpha]_D$ + 21° (*c* 0.6, $CHCl_3$). EIMS *m/z* (rel. int.): 281 ($[M]^+$, 63), 266 (8), 252 (10), 226 (27), 212 (33), 198 (100), 144 (70). ^1H NMR: δ1.00 (*t*, J = 7 Hz, 3H, H-9′), 1.45 (*m*, H-2′), 1.57 (*m*, 2H, H-4′), 2.05, (*m*, 4H, H-2′ + H-3′ + Hs-8′), 2.20 (*br*, *q*, J = 7 Hz, 2H, H-5′), 3.00 (*m*, 2H, H-1′), 3.59 (*dd*, J = 12.5 and 10.5 Hz, H-α), 4.30 (*dd*, J = 12.5 and 5 Hz, H-α), 5.35 (*m*, H-6′), 5.45 (*m*, H-7′), 6.30 (*br s*, H-3), 7.42 (*t*, J = 8.5 Hz, H-6), 7.59 (*d*,

J = 8.5 Hz, H-8), 7.68 (*td*, J = 8.5 and 1.5 Hz, H-7), 8.47 (*dd*, J = 8.5 and 1.5 Hz, H-5). ^{13}C NMR: see Table 2.

Dictyolomide B (**2**). $[\alpha]_D$ + 32° (*c* 0.9, $CHCl_3$). EIMS *m/z* (rel. int.): 299 ($[M]^+$, 1), 279 (15), 222 (5), 167 (40). ^{1}H NMR: δ0.90 (*t*, J = 7 Hz, 3H, H-9′), 1.30 (*m*, 2H, H-8′), 1.45–1.75 (*m*, 7H, H-2 + Hs-5′ + Hs-6′ + Hs-7′), 1.96 (*dd*, J = 12.5 and 4 Hz, H-2′), 2.08 (*m*, $W_{1/2} \approx$ 35 Hz, H-3′), 2.92 (*m*, 2H, H-1′), 3.60 (*m*, $W_{1/2} \approx$ 15 Hz, H-4′), 3.96 (*dd*, J = 12.5 and 10.5 Hz, H-α), 4.35 (*dd*, J = 12.5 and 5 Hz, H-α), 6.20 (*br s*, H-3), 7.36 (*t*, J = 7.5 Hz, H-6), 7.60 (*m*, H-7 + H-8), 8.40 (*d*, J = 7.5 Hz, H-5). ^{13}C NMR: see Table 2.

Acknowledgements—This work was performed in the framework of the 'Medicinal Plants of Chapare Region' project undertaken by ORSTOM and the San Simon University of Cochabamba, Bolivia.

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