

Isoquinoline Alkaloids from *Duguetia Vallicola* Stem Bark with Antiplasmodial Activity

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Received June 27, 2003; Accepted July 22, 2003

Abstract: Duguevalline (**1**), a novel aporphinoid alkaloid, in addition to four known alkaloids, cleistopholine (**2**), O-methylmoschatoline (**3**), (-)-oliveroline (**4**) and (-)-oliveridine (**5**), were isolated and characterized from *Duguetia vallicola* stems. Structural elucidation of these compounds was established by spectroscopic methods. Among them, alkaloids (**2**) and (**4**) exhibited antiplasmodial activity against *Plasmodium falciparum*.

Key Words: Isoquinoline alkaloids, *Duguetia vallicola*, Annonaceae, antiplasmodial.

INTRODUCTION

The genus *Duguetia* comprises 100 species native to tropical America, 27 of which are reported to be in the Colombian pantropical regions [1]. *D. vallicola* J.F. Macbride (Annonaceae), a large tree found in the north of Colombia, has not previously been the subject of phytochemical analyses. In this paper we discuss the isolation and structure elucidation of a new oxoaporphine, duguevalline (**1**), along with four known compounds, cleistopholine (**2**) [2, 3], O-methylmoschatoline (**3**) [4, 5], (-)-oliveroline (**4**) and (-)-oliveridine (**5**) [6, 7], from the alkaloidal extract of the *D. vallicola* stems. They were evaluated for their antiplasmodial activity against *Plasmodium falciparum*.

RESULTS AND DISCUSSION

Duguevalline (**1**) was obtained as orange needles from CHCl₃, m.p. 198-200 °C. It displayed a green spot on spraying with Dragendorff's reagent. The CIMS data showed the [M+ H]⁺ at *m/z* 366 corresponding to the molecular formula C₂₀H₁₅O₆N. An IR band at ν 1638 cm⁻¹ and a signal at δ 183.3 ppm in the ¹³C NMR spectrum indicated that a carbonyl group was present. Its UV absorption maxima at λ 205, 214, 223, 289, and 385 nm were characteristic of an oxoaporphine skeleton [8]. The ¹H NMR spectrum of (**1**) (Table 1) showed the presence of a methylenedioxy singlet at δ 6.25 assigned to positions 1 and 2, whereas three methoxy groups were identified, respectively at δ 4.24 characteristic of position 3 [9] and δ 3.98 and 3.96 located in ring D. The aromatic region of the spectrum revealed the presence of one pair of doublets at δ 8.86 and 8.12 (*J* = 5.2 Hz), assigned to H-5 and H-4 [10], and another pair at δ 7.65 and 6.83 (*J* = 2.5 Hz), which was in accord with a *meta*-substitution in ring D. The absence of

the characteristic deshielded H-11 signal indicates the MeO-9/MeO-11 substitution [11]. The unambiguous assignment of the *meta* substitution was achieved by NOESY experiments (Fig. 1). Observation of the NOESY correlations between MeO-11 and both 1,2-methylenedioxy and H-10, between MeO-9 and both H-8 and H-10, and between H-4 and both MeO-3 and H-5 protons corroborated the MeO-9/MeO-11 substitution. Further HMQC, HMBC, COSY data (Table 1) provided confirmation of structure (**1**).

The *meta*-substitution pattern in the D ring of aporphines has a taxonomic significance in the Annonaceae family. Although some aporphines with *meta*-substitution in the D ring have been reported in other families [12], these alkaloids are mainly present in the *Duguetia* and *Gutteria* genera [11, 13-18]. Thus, only one oxoaporphine with a 9,11 *meta*-substitution pattern was reported from *Gutteria discolor* [14].

Chromatographic separation also yielded cleistopholine (**2**), O-methylmoschatoline (**3**), (-)-oliveroline (**4**), and (-)-oliveridine (**5**) from the total alkaloidal extract; their structures were established by comparison of their physical and spectral data (UV, IR, ¹H NMR, ¹³C NMR, LRMS) with those published in the literature. The structures of (**4**) and (**5**) were further supported by NOESY data.

The antiplasmodial activity of the isolated compounds was assayed against *Plasmodium falciparum* sensitive strain ITG2 (Table 2).

In this study, the most active compounds were cleistopholine (**2**), and (-)-oliveroline (**4**), which showed IC₅₀ = 17.8 and 14.9 μ M, respectively.

MATERIALS AND METHODS

The UV spectra were obtained in MeOH, using a Genesys 2 (ThermoSpectronic) spectrophotometer, IR spectra were recorded on a Perkin-Elmer RXI (FT-IR). ¹H NMR (300 and 400 MHz) and ¹³C NMR (75 MHz) spectra (all in CDCl₃) were recorded with a Bruker AMX 300 and Bruker AM 400, using TMS as internal standard. The mixing time

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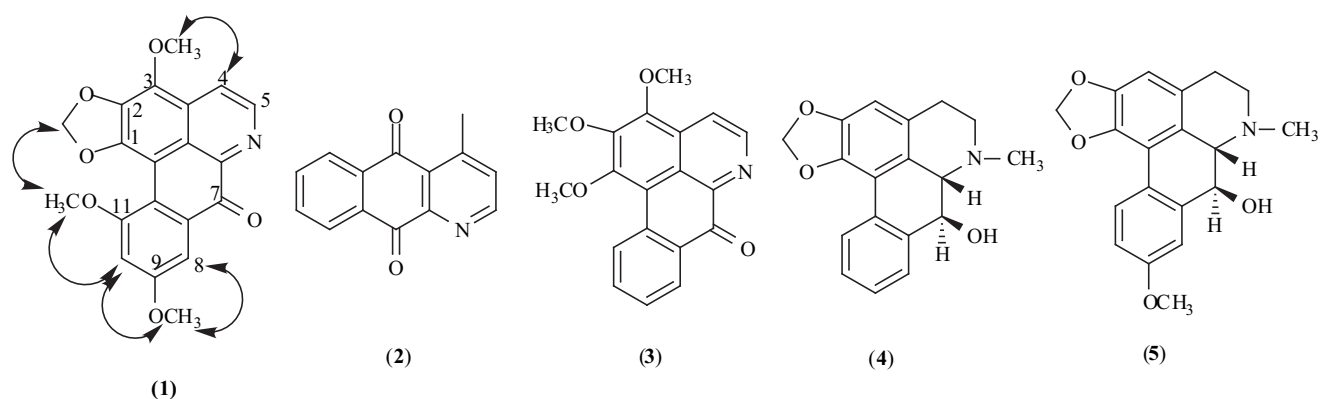


Fig. (1). Compounds (1-5) and NOESY correlations of (1).

for the HMBC spectra was 0.8 s, and the delay, in NOESY experiments, 2 s. CIMS were obtained with a Nermag-Sidar R10-10C mass spectrometer. Si gel 60 (Merck 0.063-0.200 mesh) was used for column chromatography, precoated Si gel plates (Merck 60 F₂₅₄ 0.2 mm) were used for TLC. Plates were visualized by spraying with Dragendorff's reagent or with 50% H₂SO₄ and then heating.

Table 1. Correlated ¹³C-NMR, ¹H-NMR, HMBC and COSY for Compound (1) in CDCl₃

Atom	δ_C ppm	δ_H ppm mult. J (Hz)	HMBC with H at	¹ H- ¹ H COSY
1	136.6	-	6.25	-
1a	107.5	-	-	-
1b	111.4	-	-	-
2	135.2	-	4.25	-
3	152.0	-	4.25	-
3a	121.6	-	-	-
4	118.0	8.12 d 5.2	8.86	8.86
5	144.4	8.86 d 5.2	8.12	8.12
6a	148.3	-	-	-
7	183.3	-	7.65	-
7a	133.8	-	7.65	-
8	102.7	7.65 d 2.5	6.83	6.83
9	161.6	-	6.83, 3.98	-
10	107.9	6.83 d 2.5	7.65	7.65
11	154.8	-	3.96, 6.83	-
11a	125.0	-	-	-
OCH ₂ O	101.3	6.25	-	-
MeO-3	61.8	4.25	-	-
MeO-9	55.6	3.98	-	-
MeO-11	55.2	3.96	-	-

Duguetia vallicola (Annonaceae) was collected from Montería, Colombia, in June 2001 and was identified by Dr Alvaro Cogollo. A voucher specimen (JAUM 37841) is deposited in the Jardín Botánico Joaquín Antonio Uribe, Medellín, Colombia.

Table 2. The *in Vitro* Antiplasmodial Activity of Alkaloids 1-5 Against *Plasmodium Falciparum*

Compound	IC ₅₀ (μM)
1	75.9
2	17.8
3	32.3
4	14.9
5	55.7
Chloroquine	0.06

The air-dried stems (1500 g) of *D. vallicola* were defatted by percolation with petroleum ether; the solid residue was then basified with 5% aq. NH₄OH solution and extracted with CH₂Cl₂. The combined organic extracts were then evaporated under reduced pressure. The bases were extracted with 3% aq. HCl from the CH₂Cl₂ solution. The HCl solution was basified with NH₄OH (pH 8-9) and extracted with CH₂Cl₂. The CH₂Cl₂ solution was dried over anhydrous Na₂SO₄, filtered and then evaporated to leave a brownish solid residue (17 g, 1.1%). The residue was flash chromatographed on Si gel (500 g), eluted with increasing polarities of CH₂Cl₂/MeOH mixtures. Ninety fractions of 100 mL each were collected. Fractions of similar composition (as indicated by TLC) were combined. From fractions 30-36 and fractions 37-40, cleistopholine (2, 200 mg) and O-methylmoschatoline (3, 500 mg) were obtained respectively. Fractions 41-44 (600 mg) was subjected to Si gel CC (100 g), eluting with EtOAc/MeOH (99:1). Thirty fractions were collected; fractions 5-12 furnished duguevalline (1, 50 mg). Fractions 52-56 (1200 mg), were subjected to Si gel CC (200 g), eluting with EtOAc/MeOH (96:4). Fifty fractions were thus collected. Fractions 10-25 were, subjected to preparative TLC, using hexane:EtOAc:diethylamine (8:1:1) as eluent, by which (-)-oliveroline (4, 150 mg) and (-)-oliveridine (5, 200 mg) were obtained.

Duguevalline (**1**): orange needles (CH₃Cl); m.p. 198-200 °C; UV (MeOH) λ_{\max} 205 (3.01), 214 (3.09), 223 (3.73), 289 (3.04), and 385 (2.11) nm; IR (KBr) ν_{\max} 2936, 1638, 1596, 1446, 1337, 1202 and 963 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) and ¹³C NMR (CDCl₃, 75 MHz), see Table 1. LRMS *m/z* 366 [M+H]⁺ (48), 351 (100), 334 (56), 223 (8), 308 (4) and 272 (2).

ANTIPLASMODIAL ASSAY

Antiplasmodial activity of the compounds was assessed by an *in vitro* radioisotope incorporation test using [³H]hypoxanthine [19, 20]. Each compound, plus chloroquine as a control, was assayed in triplicate at 4 different concentrations. Concentrations of both compounds tested, and positive controls, which inhibited parasite-specific incorporation of [³H]hypoxanthine by 50% (IC₅₀), were determined by non-linear regression analysis. Zero-drug controls were defined as 100% incorporation.

ACKNOWLEDGEMENTS

We thank the University of Antioquia for financial support and Pr. R. Hocquemiller for his interest in this study.

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