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(-)-8-Oxohobartine a New İndole Alkaloid from *Aristotelia chilensis* (Mol.) Stuntz

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Abstract: The fruit of *Aristotelia chilensis* is considered a "super fruit" due to its high concentration of polyphenols displaying exceptional antioxidant capacities ORAC. From maqui berries have been reported several anthocyanins and glycosylated flavonoids, those benefits increase the attention to restudy the plant. From the leaves of *A. chilensis* several indole alkaloids have been reported, we in addition to aristoteline, aristone, aristoquinoline and 3-fromylindole report the spectroscopic elucidation of 8-oxo-9-dehydromakomakine (**1**), hobartine (**2**) and a new alkaloid named 8-oxohobartine (**3**). Compound **1** to **3** did not show bactericidal activity against *E. coli* and *S. aureus* till 200 µg.

Keywords: *Aristotelia chilensis*; indole alkaloids; 8-oxohobartine.

1. Introduction

Aristotelia chilensis ([Molina] Stuntz, Elaecarpaceae) or maqui is an evergreen plant to the south of Chile, which is considered sacred for the native people due its medicinal properties. It is used particularly as an antiinflammatory agent, against kidney pains, stomach ulcers; diverse digestive ailments (tumors and ulcers), fever and cicatrisation injuries [1]. The fruit is a black berry, which have traditionally been consumed as treatment for diarrhea and dysentery. The native people from Chile, Araucanian, prepare a liquor with an ethanolic macerated solution that is used in religious ritual known as "machitun" and in daily beverages [2]. Maqui berries are well known for their high concentration of phenolic compounds, which turns maqui berries in a healthy fruit with high ORAC antioxidant capacities [3] and also displayed antimicrobial and cardioprotective activity [4].

Leaves of A. chilensis have shown anti-inflammatory, analgesic, antioxidant and antimicrobial activities [5], its chemistry has shown derivatives of non-iridoid monoterpene indole alkaloids [6] together with polyphenolic compounds. Our reinvestigation afforded in addition to previously reported quercetine, 3,5-O-dimethyl quercetine and caffeic acid ethyl ester, seven indole alkaloids including a new one called 8-oxohobartine (**3**) and two isomorphic forms of 8-oxo-9-dehydromakomakine (**1A**

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and **1B**). Structures were elucidated and discussed for related compounds by high field NMR spectroscopy.

2. Materials and Methods

General Experimental Procedures. Optical rotations were recorded on a Dichrom Model P-2000 polarimeter. FTIR spectra were measured on a Nicolet 6700 from Thermo Electron Corporation with the ATR-unit Smart Performer. Melting points were determined on a Melting Point SMP10 (Stuart) uncorrected. NMR spectra were recorded on a Bruker AVANCE III spectrometer (^{1}H: 600 MHz, ^{13}C: 150 MHz), and TMS was used as internal standard. HRESIMS were obtained using on DSQII GC/MS-system (Axel Semrau GmbH & Co). Preparative chromatography was performed using Merck silica gel 60 and Sephadex LH-20 (25−100 μm; Aldrich). Solvents used in this study were distilled prior to use and dried over appropriate drying agents.

2.1. Plant Material

Fresh leaves of *Aristotelia chilensis* (20 kg) were collected on S 36° 50’ 01.51’’ W 73° 01’ 53.75’’, Concepción, Chile, in December 2012. It was identified by Prof. Roberto A. Rodriguez (Botany Department, University of Concepción). A voucher specimen (code Ach 2012-20) has been deposited at the herbal collection of Department of Chemistry, School of Engineering and Sciences, Universidad de La Frontera, Chile.

2.2. Extraction and Isolation

The dried and milled plant material from *A. Chilensis* (8.5 kg) was extracted in acid water (30 L, pH 3, HCl) per 3 days at room temperature and filtered. The water layer was extracted with EtOAc (3 × 10 L) and the organic layer was evaporated under reduced pressure at 45 °C to afford a gummy residue (extract H^+: 52 g). The acid water was basified (pH 11, $NaHCO_3$-NaOH diluted) and extracted with EtOAc (3 × 15 L). The organic layer was evaporated under reduced pressure at 45 °C to afford a gummy residue (extract OH-: 87 g).

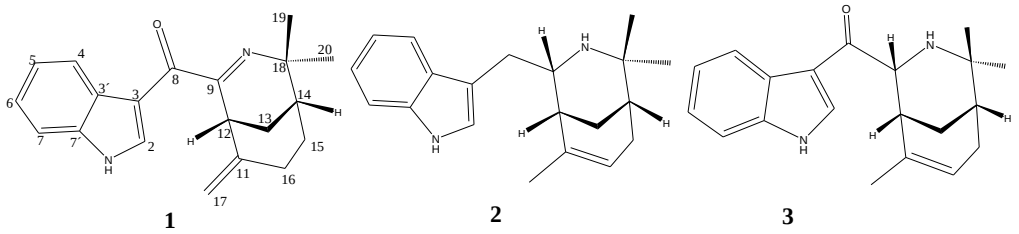
The crude H^+ extract (52 g) was chromatographed using a silica gel column and solvents (hexane 100% to ethyl acetate 100%) to give 4 subfractions A1 to A4. Fraction A2 (2.3 g) was separated by silica gel column chromatography [200−300 mesh, hex/EtOAc, 4:1] afforded caffeic acid ethyl ester (60 mg). Fraction A3 (3.3 g) was separated by silica gel CC. [200−300 mesh, hex/EtOAc, 1:1] afforded 3,5-O-dimethyl quercetine (20 mg). Fraction A4 (4.1 g) was applied to a Sephadex LH-20 column (MeOH), giving quercetine (180 mg).

The crude OH^- extract (87 g) was chromatographed using a silica gel column and solvents (hexane 100% to ethyl acetate 100%) to give 9 subfractions B1 to B9. From fractions B1 to B4 no alkaloids was obtained, only fatty acids and chlorophylls were present. Fraction B5 (4.5 g) was separated by silica gel column chromatography [200−300 mesh, hex/EtOAc, 3:1] to afford 8-oxo-9-dehydromakomakine, **1A** 130 mg; **1B** 90 mg and 3-phormyl indole 8 mg. Fraction B6 (5.2 g) was separated by silica gel CC, [200−300 mesh, hex/EtOAc, 1:1] afforded Aristone, 10 mg. Fraction B7 (6.6 g) was applied to a Sephadex LH-20 column (EtOAc), giving aristoteline 210 mg. Fraction B8 (2.1 g) was applied to a Sephadex LH-20 column (EtOAc) and further separated by silica gel CC [200−300 mesh, EtOAc 100%] afforded hobartine 60 mg and 8-oxohobartine 12 mg.

3. Results and Discussion

3.1. Structure elucidation

Basic extracts of leaves of *Aristotelia chilensis* were purified by CC on silica gel and Sephadex LH 20 giving in addition to aristone, aristoteline, 3-formylindole, aristoquinoline, two isomorphic compounds of 8-oxo-9-dehydromakomakine (**1**), hobartine (**2**) and a new alkaloid called 8-oxohobartine (**3**). (Figure 1).



1 **2** **3**

Figure 1. Structures of 8-oxo-9-dehydromakomakine (**1**), hobartine (**2**) and 8-oxohobartine (**3**).

The less polar alkaloid was purified by silica gel column chromatography (hex/EtOAc, 3:1). It was identified as 8-oxo-9-dehydromakomakine (**1**), based on HRMS-ESI $[M + H]^+$ *m/z* 307.1654 and ^{13}C NMR data. The molecular formula $C_{20}H_{22}N_2O$ was assigned. Crystallization of **1** affords pale-yellow crystals (figure 2A). Additionally, another compound was isolated **1B**, of which crystals were deep-red colored (figure 2B). HRMS-ESI $[M + H]^+$ *m/z* 307.1748 and ^{13}C NMR data show a molecular formula $C_{20}H_{22}N_2O$. NMR spectroscopic data elucidated **1B** as 8-oxo-9-dehydromakomakine, which was previously reported [6]. Color differences for compounds **1A**–**1B** can be explained due to a different torsion angle between the indolyl ketone system and the planar portion of the heterocyclic six-membered ring (O=C8-C9=N angle, 130.7 in the yellowish **1A** and 161.6 in the deep-red **1B,** from X-ray). These angle differences would cause variation in the electronic conjugation [7]

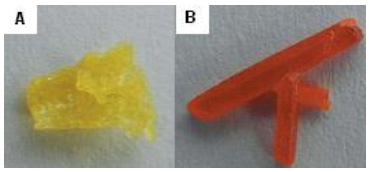


Figure 2. Polymorphic form of 8-oxo-9-dehydromakomakine **1**. (A) pale-yellow crystal of (**1A**) and (B) a deep-red crystal of (**1B**).

IR of **1A** and **1B** show differences around the conjugated central system, for **1A** the absorption N-H is a broad signal at 3248 cm^{-1}, C=O 1589 $cm^{-1}$and N=C is a small absorption at 2431cm^{-1}, for **1B**, N-H is a small signal at 3253 cm^{-1}, the carbonyl group remain at C=O 1588 cm^{-1} and N=C is a small absorption at 2431 cm^{-1}. The melting point also show the difference between both molecules: for **1A** mp is 259-260°C meanwhile for **1B** is 256-257°C OR is $[\alpha]_D^{25}$: +7.9.

^1H NMR data (Table 1) show characteristic resonances of two methyl groups, a singlet proton H-2 at 8.07 ppm of the indole ring and two olefinic protons, while ^{13}C NMR and HSQC data (Table 2) show 20 carbon resonances including a ketone at C-8, two methyl groups at C-18 and the exo-methylene group at C-11. Similarly, HMBC data of 1 indicate that Me-19 (δH 1.52) correlates with C-9/C-20/C-14, while H-14 (δH 1.89) correlates with C-18 and C-15. H-17a and b (δH 4.75/4.70) correlate with C11/C13 and only H-17a (δH 4.75) correlates with C-12. H-14 (δH 1.89) correlates with C-18 and C-15. The $^1H-^1H$ COSY data show correlation of H-14 with each one proton of H-15 and H-13. H-12 correlates also with the same H-13. H-16a-b overlap with other signals which makes it impossible to determine their orientation.

From fraction B8 (2.1 g) it was purified through Sephadex LH-20 column and silica gel CC, 60 mg of **2**. On the basis of HRMS-ESI $[M + H]^+$ *m/z* 295.2200 and ^{13}C NMR data, **2** has a molecular formula $C_{20}H_{26}N_2$. Its IR absorption bands at 3146 and 1618 cm^{-1} indicate the presence of amino and alkenyl groups. Its ^1H NMR data (Table 1) show characteristic resonances of three methyl groups, one of them at 1.74 ppm and a singlet proton H-2 at 7.07 ppm of the indole ring and four protons in the aromatic region. Its ^{13}C NMR and HSQC data (Table 2) show 20 carbon resonances including three methyl groups, three CH_2, nine CH and five quaternary carbons. Protons were assigned to related carbon resonances via the ^1H-NMR and HSQC spectroscopic data. The $^1H-^1H$ COSY data of **2**

exhibits correlations of H-16 with Me-17/H-15a/H-15β. H-12 correlates with H-9/H-13. H-14 correlates with H-15a. Similarly, its HMBC data indicate that Methyl groups 19 and 20 correlate with C-18, but only Me-19 correlates with H-9/H-14/H-13.

From the same fraction B8 (2.1 g), after hobartine was purified from silica gel CC, 12 mg of compound **3** were isolated. HRMS-ESI positive-ion $[M + H]^+$ *m/z* 309.1962 and ^{13}C NMR data, show that **3** has the molecular formula $C_{20}H_{24}N_2O$. Its IR absorption bands at 3011, 2973, 1626, 1525 cm^{-1} indicate the presence of amino, carbonyl and alkenyl groups. Its ^1H NMR data (Table 1) show characteristic resonances of three methyl groups, and a singlet proton H-2 at 8.37 ppm, four protons in the aromatic region and two olefinic protons at δ5.58 and 4.68 ppm. Its ^{13}C NMR and HSQC data (Table 2) show 20 carbon (as also found for the other Aristotelia alkaloids) which include three methyl groups, two CH_2, nine CH and six quaternary carbons. On the basis of these NMR data and the structures of the alkaloids previously isolated from *A. chilensis*, compound **3** was tentatively assigned as an alkaloid related to hobartine. The $^1H-^1H$ COSY data of **3** exhibit correlations between H-14 and H-13a/H-15a. Me-17 correlated with H-16/H-15ab. H-9 correlated with Me-19/H-12/H-2. The HMBC data indicate that Me-17 (δH 1.31) correlates with C-12/C-11/C-16. Me-19 (δH 1.33) correlates with Me-20/C-18/C-14. H-9 (δH 4.68) correlates with C-8/C-12/C-11/C-13. H-13a (δH 1.70) C-8/C-11/C-9/C-18/C-12/C-14/C-15. These results indicated that **3** is a C-8 ketohobartine. The relative configuration of **3** was determined by analyzing its NOESY data. The α -orientation of H-9 was indicated by its NOE's with H-12, H-2 and Me-19.

Table 1. ^1H NMR data for compounds **1-3** (at 600 MHz in CD_3OD, δ in ppm, *J* in Hz).

H	1	2	3
2	8.07, *s*	7.07, *s*	8.37, *s*
4	8.28, *m*	7.54, *dt*, *J* = 7.9, 0.9	8.20, *ddd*, *J* = 7.8, 1.3, 0.8
5	7.25, *m*	7.00, *ddd*, *J* = 8.0, 7.0, 1.0	7.20, *ddd*, *J* = 7.9, 7.0, 1.1
6	7.25, *m*	7.08, *m*	7.24, *ddd*, *J* = 8.1, 7.0, 1.2
7	7.46, *m*	7.33, *dt*, *J* = 8.1, 0.8	7.47, *ddd*, *J* = 8.0, 1.0, 1.0
8	-	2.72, *m*	-
9	-	3.51, *ddd*, *J* = 8.0, 6.8, 2.4	4.68, *d*, *J* = 2.9
12	3.77, *m*	2.19, *dd*, *J* = 5.6, 2.9	2.54, *dd*, *J* = 5.7, 2.8
13	2.25, *m*	1.57, *dt*, *J* =12.6, 3.2	1.70, *dt*, *J* = 12.5, 3.3
	1.77, *ddd*, *J* = 12.5, 3.2, 2.3	2.05, *m*	2.47, *dt*, *J* = 12.5, 2.4
14	1.89, *m*	1.44, *ddd*, *J* = 6.2, 3.1, 3.1	1.56, *ddd*, *J* = 6.1, 3.0, 3.0
15	2.17, *m*	2.27, *d* (br), *J* = 19.0	2.13, *m*
	1.64, dddd, *J* = 13.4, 13.4, 6.1, 4.1	2.06, *m*	2.31, *d* (br), 18.1
16	2.26, *m*	5.60, *s* (br)	5.58, *s* (br)
17	Ha 4.75, *t*, *J* =1.8	1.75, *dd*, *J* = 4.0, 1.7	1.3, *s* (br)
	Hb 4.70, *t*, *J* = 1.7		
19	1.52, *s*	1.20, *s*	1.33, *s*
20	1.32, *s*	1.10, *s*	1.22, *s*

8-Oxo-9-dehydromakomakine (1): red crystals (MeOH); mp= 256-257°C; $[\alpha]_D^{25}$: + 7.9 (c: 0.24, $CHCl_3$); IR *ν*max 3253, 3152, 2969, 2938, 2862, 2425, 1588, 1503, 1448, 1360, 1206, 960, 890, 742 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRMS-ESI $[M + H]^+$ *m/z* 307.1748 (calcd for C20H23N2O, 307.1732).

Hobartine (2): colorless crystals (MeOH); mp= 150-151°C; $[\alpha]_D^{25}$: -25.16 (c: 0.225, $CHCl_3$); IR *v*max 3146, 2932, 1618, 1450, 1357, 1230, 1098, 733 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRMS-ESI $[M + H]^+$ *m/z* 295.2200 (calcd for C20H27N2, 295.2174).
8-Oxohobartine (3): colorless crystals (MeOH); $[\alpha]_D^{22}$: -75.7 (c 0.04, $CHCl_3$); IR *v*max 3011, 2973, 2912, 1626, 1525, 1447, 1378, 1155, 750 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRMS-ESI $[M + H]^+$ *m/z* 309.1962 (calcd for $C_{20}H_{25}N_2O$ 309.1961).

Table 2. ^{13}C NMR data for compounds **1-3** (at 150 MHz in CD_3OD δ in ppm).

C	1	2	3
2	138.4	123.6	134.4
3	115.8	113.0	116.3
3´	127.6	128.8	127.1
4	123.0	119.4	122.7
5	123.7	119.5	123.3
6	124.7	122.3	124.4
7	113.0	112.3	113.0
7´	138.3	138.2	138.5
8	190.3	31.8	196.4
9	169.2	55.6	60.6
11	147.4	134.4	133.5
12	42.1	38.1	41.6
13	30.3	30.0	29.3
14	37.1	36.5	35.6
15	30.9	28.8	28.3
16	30.0	126.1	125.6
17	110.6	26.0	24.0
18	60.3	54.9	54.1
19	27.0	25.8	25.7
20	31.3	29.8	29.5

3.1.2. Paper disc diffusion assay

The antibacterial activity was evaluated using the disc diffusion method [8]. The compounds **1** to **3** were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 1000 µg/mL. Sterile paper discs of 6 mm in diameter were impregnated with 50 µg, 100 µg and 200 µg of each compound and deposited on the agar surface previously inoculated with 250 µL of bacterial cell suspension (*S. aureus* or *E. coli*), the plates were incubating at 37 °C in the dark for 24 h. The diameter of an inhibition zone around the discs was measured after incubating. DMSO was used as a negative control and 20 µg of kanamycin was used as a positive control for this assay. The three compounds were inactive against *E. coli* and *S. aureus* till 200 µg, the inhibition halo was in order to DMSO. This assay was done in 3 replicates.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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