

Extractives from *Heteropyxis natalensis*

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Abstract

(2E)-2-[(2E)-1-hydroxy-3-phenylprop-2-en-1-ylidene]-5-methoxy-6,6-dimethylcyclohex-4-ene-1,3-dione (ceroptin), and two other flavonoids have been isolated from the leaf extract of *Heteropyxis natalensis*. Their structures were determined using both 1D- and 2D-NMR as well as X-ray diffraction studies.

Keywords: *Heteropyxis natalensis*; Heteropyxidaceae; ceroptin, flavonoids, x-ray analysis

Introduction

The family Heteropyxidaceae is a small family with only three species in southern Africa namely *Heteropyxis canescens*, *H. dehniae*, and *H. natalensis*, commonly known as Lavender tree or Laventelboom, ranges from Zimbabwe through the Northern Province, Mpumalanga and KwaZulu-Natal of South Africa. It is a slender, upright tree which grows to about 10 meters high.¹ It is recorded as a Zulu medicinal plant used as medicinal tea. Its bark is used to treat impotence and as an aphrodisiac.²

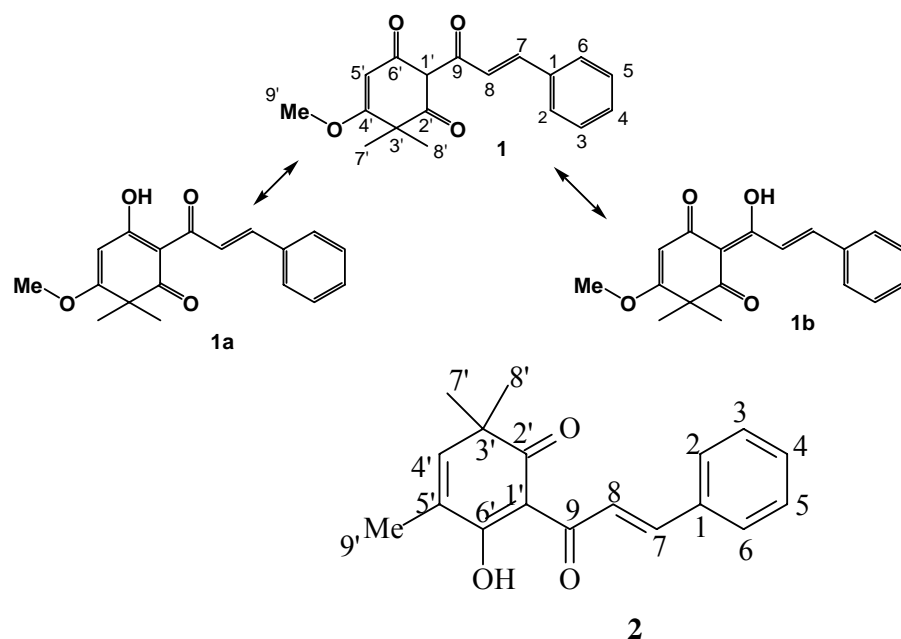
Previously, several investigators have examined the essential oils from the leaves of *H. natalensis*.³ In our on-going search for bioactive natural products as leads for new ethnopharmaceuticals, *H. natalensis* was subjected to phytochemical examination. Our preliminary results are reported herein.

Results and Discussion

The chromatographic fractionation of the hexane, dichloromethane, and ethyl acetate extracts of the leaves of *H. natalensis* afforded compounds **A**, **B**, **C**, and **D** as major extractives as well as two minor constituents.

The ¹³C NMR spectrum of compound **A** (yellow crystalline compound mp. 136)(Table 1) shows 18 signals including two signals at δ_{CH} 144.63 and 123.38 characteristic of the β and α carbons of a chalcone structure⁴, seven sp^2 CH (δ 30.52, 128.87, 130.52, 123.38, 95.58, and 56.55), one sp^2 quaternary carbon (δ 180.08), four sp^3 carbons (δ 56.55, 1 x OCH₃, δ 49.27, 1 x C(CH₃)₂, δ 24.67, 2 x CH₃), and three carbonyl groups (δ 197.60, 190.93, and 187.78). The ¹H NMR spectrum (Table 1) showed two signals corresponding to five aromatic protons at δ 7.65 (2 x ArH) and δ 7.36 (3 x ArH), two doublets at δ 8.30 (1 x H, 15.9 Hz) and δ 7.89 (1 x H, 15.8 Hz) attributable to *trans* olefinic protons, one downfield singlet at δ 5.48, a singlet at δ 3.81 (1 x OCH₃), a singlet at δ 3.74 (1 x olefinic proton), and a singlet at δ 1.38 (2 x CH₃).

These data are in agreement with a chalcone structure in which ring A is non-aromatic with three oxygen functions and unsubstituted ring B. The molecular formula, C₁₈H₁₈O₄, which was obtained by HREIMS m/z [M⁺, 100] 298.1234 (calc. 298.1205 for C₁₈H₁₈O₄) led us to propose structure **1** for compound **A**.



A review of chemical literature for structure **1** revealed that a compound of identical structure, named ceroptene, was isolated from *Pityrogramma triangularis*.⁵ However, subsequent workers⁶ renamed ceroptene as ceroptin and confirmed the structure as **1a**, the enol isomer of **1**. The structure of compound **A** was finally confirmed by X-ray crystallographic analysis as (2E)-2-[(2E)-1-hydroxy-3-phenylprop-2-en-1-ylidene]-5-methoxy-6,6-dimethylcyclohex-4-ene-1,3-dione (Fig. 1) which is the resonance hybrid of **1**, **1a**, and **1b**. It is pertinent to mention that the spectral data (Table 2) of ohobanin (**2**), isolated from *Oreopteris quelpaertensis*⁷, compare very well with those of compound **A**.

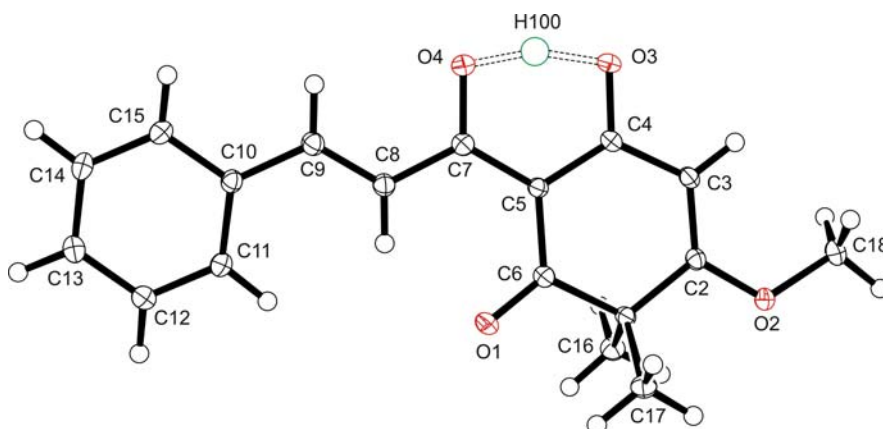


Figure 1: X-ray structure of compound **A**.

Table 1: ¹³C and ¹H NMR Spectral data of Compound A

Position	C atom	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm), J (Hz)
1	C	135.26	
2,6	CH	128.83 ^a	7.65 <i>m</i>
3,5	CH	128.88 ^b	7.36 <i>m</i>
4	CH	130.70	
7	CH	144.63	7.89 <i>d</i> , 15.80
8	CH	123.38	8.30 <i>d</i> , 15.90
9	C	187.78	
1'	CH	56.55	3.47 <i>s</i>
2'	C	197.60	
3'	C	49.27	
4'	C	180.08	
5'	CH	95.58	5.48 <i>s</i>
6'	C	190.93	
7'	CH ₃	24.67	1.38 <i>s</i>
8'	CH ₃	24.67	1.38 <i>s</i>
9'	CH ₃	56.55	3.81 <i>s</i>

Table 2: ¹³C NMR and ¹H NMR of Ohobanin (2)

Position	C atom	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm), J (Hz)
1	C	135.12	
2,6	CH	128.88 ^a	7.70 – 7.66 <i>m</i>
3,5	CH	128.81 ^b	
4	CH	130.13	7.40 – 7.38 <i>m</i>
7	CH	145.09	8.30 <i>d</i> , 15.80
8	CH	123.76	7.95 <i>d</i> , 15.80
9	C	187.72	
1'	CH	108.35	
2'	C	199.95	
3'	C	45.97	
4'	C	151.77	6.52 <i>q</i> , 1.32
5'	CH	129.24	
6'	C	190.99	18.88 <i>s</i> , (OH)
7'	CH ₃	26.41	1.30 <i>s</i>
8'	CH ₃	26.41	
9'	CH ₃	15.46	1.99 <i>d</i> , 1.32

The ¹³C NMR spectrum (Table 3) of compound **B** (orange yellow crystalline compound, mp 139-140) showed 17 signals including two signals at δ^{CH} 143.27 and 126.38 corresponding to the β and α carbons of chalcone⁴, six sp^2 CH at δ 130.30, 128.95, 128.45 corresponding to six aromatic carbons (five in the same ring), six sp^2 quaternary carbons at δ 164.13, 161.51, 161.45, 135.28, 110.25, and 109.55, two sp^3 carbons at δ 62.24 (1 x OCH₃) and δ 8.04 (1 x CH₃), and one carbonyl at δ 193.17. The ¹H NMR spectrum showed a downfield signal at δ 13.28 indicating the presence of a chelated hydroxyl, which was confirmed by IR spectrum

Experimental

Melting points (uncorrected) were determined on a Stuart Scientific SMP1 apparatus. IR spectra (KBr) were recorded on a Nicolet Impact 420 spectrophotometer. NMR spectra (both 1D and 2D) were obtained on a Varian 300 (300 MHz) spectrometer, using the residual solvent peaks as internal standards. HR-EIMS and GC-MS were determined on a Kratos 9/50 HRMS instrument and a MAT Finnigan GCQ spectrometer, respectively. Column chromatography was carried out using Merck Si gel 60 (70-230 mesh). Analytical TLC was carried out on precoated aluminum plates using Merck Si gel F254; plates were visualized under UV light (λ 254 and 366 nm) and by spraying with anisaldehyde/H₂SO₄ reagent, followed by gentle heating.

Plant material

Fresh leaves of *H. natalensis* were collected in October 2004 from Durban and identified by H. Baijnath. A voucher specimen (MH/06) was deposited in the Wards Herbarium, UKZN-Westville Campus

Extraction and isolation

The powdered air-dried leaves (1.1kg) of *H. natalensis* were successively extracted by maceration at room temperature in hexane, dichloromethane, ethyl acetate, and methanol to give, after removal of solvent *in vacuo*, hexane extract (19.1g), DCM extract (39.6g), ethyl acetate extract (16.5g) and methanol extract (126.5g).

Column chromatography of the hexane extract (3g) over silica gel using hexane/ethyl acetate (5-10% of ethyl acetate) as eluant, gave compound **A** (160mg) and compound **D**. Similarly, dichloromethane extract (3g) gave compound **A** (250mg), compound **B** (105mg), and compound **C** (240mg).

Column chromatography of the ethyl acetate extract (3g) over silica gel using hexane/ethyl acetate (5-30% of ethyl acetate) as eluant, gave compound **C** (360mg).

Compound A (Yellow crystalline compound)(3.9%).

Melting point: 136°C

UV (CHCl₃): λ_{\max} 370 nm

IR (KBr): ν_{\max} /cm⁻¹ : 1646, 1631, 1573, 1525, 1432, 1372, 1229, 1161, 987, 880

HREIMS: m/z [M⁺, 100] 298.1234 (calc. 298.1205 for C₁₈H₁₈O₄).

¹H NMR and ¹³C NMR. See Table 1.

X-Ray crystal structure analysis of Compound A:

Empirical formula	C ₁₈ H ₁₈ O ₄	
Formula weight	298.32	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	$a = 8.3050(8)$ Å	$\alpha = 112.525(9)^\circ$
	$b = 9.7896(8)$ Å	$\beta = 92.828(9)^\circ$
	$c = 10.0259(12)$ Å	$\gamma = 94.058(7)^\circ$
Volume	748.47(13) Å ³	
Z	2	
Density (calculated)	1.324 Mg/m ³	

Absorption coefficient	0.093 mm ⁻¹
F(000)	316
Crystal size	0.38 x 0.20 x 0.10 mm ³
Theta range for data collection	4.69 to 25.05°
Index ranges	-9<=h<=9, -11<=k<=11, -11<=l<=11
Reflections collected	7399
Independent reflections	2604 [R(int) = 0.0183]
Completeness to theta = 25.00°	98.4 %
Absorption correction	None
Max. and min. transmission	0.9908 and 0.9655
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	5073 / 0 / 238
Goodness-of-fit on F ²	1.254
Final R indices [I>2sigma(I)]	R1 = 0.0634, wR2 = 0.1546
R indices (all data)	R1 = 0.0678, wR2 = 0.1574
Largest diff. peak and hole	0.567 and -0.293 e Å ⁻³

Compound B: (orange yellow crystalline compound) (0.13%)

Melting point: 139-140°C

IR (KBr): ν_{\max} /cm⁻¹: 3252 (br OH), 3106, 3048, 29, 1631, 1505, 1413

HREIMS: m/z [M⁺, 100] 284.10408 (calc. 284.10486 for C₁₇H₁₆O₄).

¹H NMR and ¹³C NMR. See Table 3.

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