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Megistosarcimine and megistosarconine, two alkaloids from *Sarcomelicope megistophylla*

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Abstract

Two new alkaloids, megistosarcimine and megistosarconine, were isolated from the aerial parts of *Sarcomelicope megistophylla*. Their structures have been elucidated on the basis of their spectral data and molecular modeling.

Keywords: *Sarcomelicope megistophylla*; Rutaceae; Alkaloids; Tetrahydrofuroquinoline; Megistosarcimine; Megistosarconine; Cytotoxicity

1. Introduction

Sarcomelicope megistophylla Hartley (Rutaceae) is a small to medium tree, 8–12 m high, easily recognized by its large pubescent leaves. Hartley (1986) recently described it as endemic to the region of Néaoua, New Caledonia.

In a continuation of our chemical studies of the genus *Sarcomelicope* (Baudouin et al., 1985; Brum-Bousquet, Mitaku, Skaltsounis, Tillequin, & Koch, 1988; Brum-Bousquet, Tillequin, Koch, & Sevenet, 1985; Mitaku & Pusset, 1988; Mitaku, Skaltsounis, Tillequin, Koch, & Pusset, 1986a; Mitaku et al., 1986b; 1987; 1995; Skaltsounis et al., 1995) we report here the structural elucidation of two minor alkaloids isolated from the aerial parts of *Sarcomelicope megistophylla*, whose major alkaloids have been already described (Skaltsounis et al., 1995). The ^{1}H and ^{13}C NMR spectral data of the two novel alkaloids were assigned by interpretation of COSY 45°, COSY-LR, DEPT 135°, HMQC, HMQC-TOCSY and HMBC experiments.

2. Results and discussion

Megistosarcimine (**1**) was obtained as a colorless amorphous compound. Its empirical formula was determined by high-resolution mass spectroscopy as $C_{18}H_{20}N_2O_4$ (Found: 328.1425, calcd: 328.1423). The UV spectrum was suggestive of a tetrahydrofuroquinoline derivative. The IR spectrum showed the presence of a ketonic carbonyl group (1730 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed the presence of two furanic protons, (δ 7.65 and 7.05, two d, J = 3 Hz), two methoxy groups, one aromatic (δ 4.45) and one aliphatic (δ 3.32), a methylene group AB system (δ 2.22, d, J = 15 Hz and 2.10, dd, J = 15 Hz, J' = 1.7 Hz), a CH_2CH AMX system (δ_A 2.55 and δ_M 2.83, two dd, J = 14 Hz, J' = 3.5 Hz; δ_X 2.94, td, J = 3.5 Hz, J' = 1.7 Hz) and two C- methyl groups (δ 1.19 and 1.10). The ^{13}C NMR (Table 2) spectrum exhibited the signals of one highly deshielded carbonyl, one imine,

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Table 1
^{1}H NMR spectra of compound **1**, **3** and **2** in $CDCl_3$, δ in ppm, J in Hz

	1	**3**	**2**
H-6	2.94, td, $J = 3.5$, $J' = 1.7$	2.80, td, $J = 3.2$, $J' = 1.7$	2.82, t, $J = 4$
H-7a	2.83, dd, $J = 14$, $J' = 3.5$	2.91, dd, $J = 13.5$, $J' = 3.2$	2.96, dd, $J = 14$, $J' = 4$
H-7b	2.55, dd, $J = 14$, $J' = 3.5$	2.60, dd, $J = 13.5$, $J' = 3.2$	2.51, dd, $J = 14$, $J' = 4$
H-2′	7.65, d, $J = 3$	7.67, d, $J = 3$	7.63,d, $J = 3$
H-3′	7.05, d, $J = 3$	7.11, d, $J = 3$	7.02, d, $J = 3$
H-2a″	2.22, d, $J = 15$	2.38, d, $J = 15$	2.22, d, $J = 15$
H-2b″	2.10, d, $J = 15$, $J' = 1.7$	2.20, d, $J = 15$, $J' = 1.7$	2.12,d, $J = 15$
CH_3-C-3″	1.19, s	1.21, s	1.12, s
CH_3-C-3″	1.10, s	1.10, s	1.10, s
OCH_3-C-4	4.45, s	4.45, s	4.33, s
OCH_3-C-5	3.32, s	3.38, s	3.38, s
NH-C-8	3.89, br s	–	–
$NCOCH_3$-C8	–	–	2.32, s

two tetrasubstituted, one trisubstituted and two disubstituted sp^3 carbons, five quaternary and two tertiary sp^2 carbons, two C-methyl and two O-methyl groups. The 4-position of the most deshielded methoxy group (4.45) was deduced from a COSY LR experiment where a clear cross peak between the OCH_3 and the H-3′ (7.05) was observed. These elements permitted to envisage the presence of a tetrahydrofuro[2,3b]quinoline fused to a cyclopentanone ring. Further information on the structure of **1** was obtained from the results of the long range C-H correlation in the HMBC spectrum (Table 2 and Fig. 1). Appearance of three and two bond correlations between the two CH_3 at $\delta_H = 1.19$ and 1.10 ppm and the 6-tertiary carbon at $\delta_c = 55$ ppm and the 3″-quaternary carbon at $\delta_c = 37.8$ ppm respectively, permitted localization of the dimethyl group at position C-3″ of the cyclopentanone ring. Appearance of three and two bond correlations between the CH_2 at $\delta_H = 2.94$ and 2.55 ppm, and the 8a-quaternary carbon at $\delta_c = 154.8$ ppm and the imine carbon (C-8) at $\delta_c = 171.5$ ppm, on one hand, and of three and two bond correlations between the CH_2 at $\delta_H = 2.22$ and 2.10 ppm, and the 5-quaternary carbon at $\delta_c = 84$ ppm, and the carbonyl carbon at $\delta_c = 203.9$ ppm on the other hand, permitted localization of the dimethylcyclopentanone ring at position 5 and 6 of the tetrahydrofuroquinoline system. The presence of a free NH-group was confirmed by formation of a monoace-

Table 2
^{13}C NMR spectra of compound **1** and **3** in $CDCl_3$, δ in ppm and HMBC correlations

	1		3	
Position	δ ^{13}C	HMBC	δ ^{13}C	HMBC
C-2	163.7		164.9	
C-3	106.9	H-2′	107.2	H-2′
C-4	161.7	OCH_3-C-4	163.5	OCH_3-C-4
C-4a	113.8		115.8	
C-5	84.0	H_a-2″, H_b-2″	83.9	H_a-2″, H_b-2″
C-6	55.0	$(CH_3)_2$-C-3″ H_a-2″, H_b-2″	57.4	$(CH_3)_2$-C-3″ H_a-2″, H_b-2″
C-7	32.4		32.5	
C-8	171.5	H_a-7, H_b-7	196.2	H_a-7, H_b-7
C-8a	154.8	H_a-7, H_b-7	159.2	H_a-7, H_b-7
C-2′	144.2	H-3′	143.9	H-3′
C-3′	105.8	H-2′	106.6	H-2′
C-1″	203.9	H_a-2″, H_b-2″	202.8	H_a-2″, H_b-2″
C-2″	49.9		49.7	
C-3″	37.8		37.4	
CH_3-C-3″	28.6		28.5	
CH_3-C-3″	28.6		28.5	
OCH_3-C-4	59.6		59.9	
OCH_3-C-5	51.5		51.6	
N*CO*CH_3-C8	–		–	
NCO*CH_3*-C8	–		–	

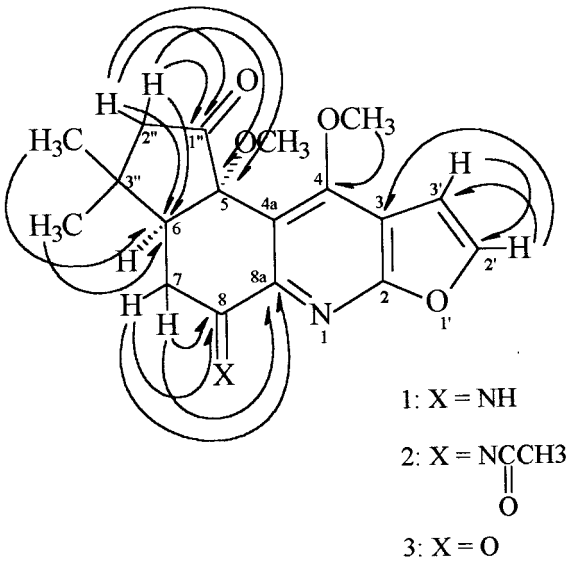
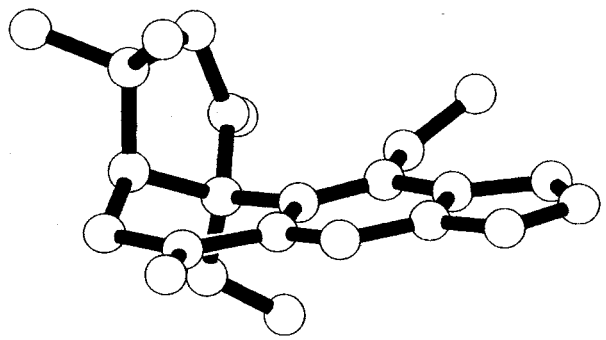
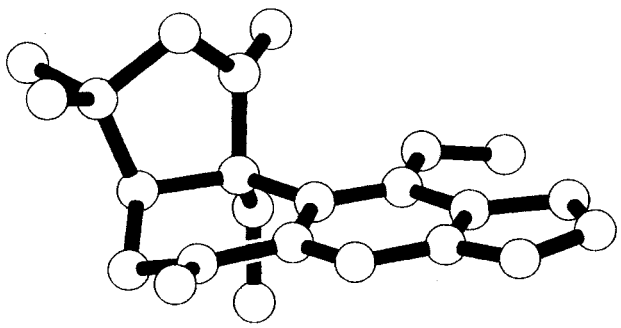


Fig. 1. Structure of compounds **1**, **2**, **3** and HMBC correlations of **1** and **3**.

tyl derivative **2** upon acetylation under mild conditions (Ac_2O-pyridine, rt 24 h). The relative configuration between the C5-OMe and the C6-H could be deduced as *cis* from NOESY experiments, coupling constants and molecular mechanics consideration. The 2D phase sensitive NOESY spectrum (mixing time 700 ms) showed that the proton H-6 correlated with a small cross peak with the one methyl group on C-3″ (1.1 ppm) and a more intense cross peak with the other methyl group on C-3″ (1.19 ppm). This methyl group correlates with H-7b and H-2b″. The other methyl group on C-3″ (1.1 ppm) correlates with both protons H-7a and 7b. Considering also that the H-6 has the same ^3J coupling constant (3.5 Hz) with both H-7 protons we proceed in a Monte-Carlo conformational search using the Macromodel software (Macromodel 5.0, MM2*, 1000 steps). Two different conformational searches have been performed, one for the '*cis*' stereochemistry and one for the '*trans*'. The results show that in the '*trans*' configuration none of the conformers generated by Monte-Carlo has the H-6 gauche with both the H-7 protons. In the case of the '*cis*' stereochemistry two different low energy conformers (Fig. 2) agree with the NOESY spectrum and the observed *J* coupling constants between H-6 and H-7a and H-7b protons. Nevertheless, the absolute configuration of the chiral centre at C-5 and C-6 could not be determined due to the small amount of natural product isolated.



E rel = 0 kcal/mol



E rel = 0.3 kcal/mol

Fig. 2. The two minimal energy structures for compound **1**. The internal coordinates of the molecules were created using the molecular graphics of the HYPERCHEM software.

Megistosarconine (**3**) was obtained as a colorless amorphous compound. The empirical formula was determined by high resolution mass spectroscopy as $C_{18}H_{19}NO_5$ (found: 329.1260, calcd.: 329.1263). The UV spectrum similar to that of **1** was also suggestive of a tetrahydrofuroquinoline derivative. The IR spectrum showed the presence of one ketonic and one conjugated ketonic carbonyl group (1735 and 1685 cm^{-1}). Comparison of the ^1H and ^{13}C NMR spectral data (Table 1 and 2) of **3** with those of **1** indicated that in **3** the imino group was replaced by a carbonyl group. The COSY-LR, NOESY and HMBC spectra exhibited the same cross peak as those observed in the case of megistosarcimine. Finally NOESY studies and molecular mechanics calculation have also deduced the relative *cis* configuration between the C_5-OMe and the H-6.

Its important to point out that megistosarcimine (**1**) is very instable and transformed in fact in the course of the separation procedure into megistosarconine (**3**). Indeed treatment of **1** with water yielded in few hours, quantitatively **3**. In contrast treatment of megistosarconine (**3**) by an ammoniacal solution 20% (extraction conditions) left **3** intact. So an artificial formation of **1** can be excluded. Although tetrahydrofuroquinoline derivatives have been already reported in the literature (Al-Yahya, El-Domiaty, Al-Meshal, Al-Said & El-Feraly, 1991), this is the first time that a tetrahydrofuroquinoline fused to a cyclopentane ring is described in a natural product.

The most stable compound, megistosarconine was evaluated for its cytotoxic activity against L 1210 leukemia cells and showed a moderate activity ($IC_{50} = 70$ μM).

3. Experimental

3.1. General

UV: MeOH; NMR: Bruker DRX 400, Bruker AC 200 spectrometers [^1H (400 and 200 MHz) and ^{13}C (50 MHz)], with TMS was the int. standard. Chemical shifts are reported in δ (ppm) values. The 2D NMR experiments were performed using standard Bruker microprograms. EIMS and DICMS (using NH_3 as reagent gas): Nermag R 10-10C spectrometer. HRMS: AEI MS-902 spectrometer. CC: silica gel Merck 0.04–0.06 mm (flash) and silica gel 0.015–0.04 mm. The geometry optimization was performed using the Polack–Ribiere conjugate gradient with a termination condition of 0.1 kcal/mol.

3.2. Plant material

The plant material was collected at Néaoua (New Caledonia) in May 1984. Herbarium samples (Pusset-Chauviere 261) are held in the herbaria of the Centre ORSTOM de Noumea and of the Museum National d'Histoire Naturelle (Paris).

3.3. Cytotoxicity assay

The murine leukemia L1210 was from the American Type Culture Collection (Rockville Pike, MD). Cells were grown in RPMI medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by the Microculture Tetrazolium assay as previously described (Mitaku et al., 1996).

3.4. Extraction and isolation of alkaloids

Extraction of alkaloids as described (Mitaku et al., 1986b) The crude alkaloid mixture was chromatographed over a CC silica gel Merck 0.04–0.06 mm (flash), using CH_2Cl_2-MeOH gradient. Fraction 100–120 was chromatographed with MPLC (silica gel 0.015–0.04 mm, hexane-EtOAc gradient) to afford **3**, (25 mg) and **1** (17 mg).

3.5. Megistosarcimine (*1*)

$[\alpha]_D + 131^\circ$ (c 0.2, $CHCl_3$); UV λ_{max} (log ε) (MeOH) 252 (4.25), 275 sh.; IR ν_{max} cm^{-1}: 1605, 1730, 2940, 3150, 3160; ^1H and ^{13}C NMR see Tables 1 and 2; DCIMS m/z 329 $[M+H]^+$; EI m/z (rel.int.): 328 (33), 313 (100), 285 (70), 243 (76), 229 (70).

3.6. Acetylation of *1*

Treatment of **1** (10 mg) with Ac_2O (1 ml) in pyridine (1 ml) at room temp followed by flash CC (hexane-EtOAc) gave the monoacetate **2**. DCIMS m/z 371 $[M+H]^+$; ^1H NMR see Table 1.

3.7. Megistosarconine (*3*)

$[\alpha]_D + 162^\circ$ (c 0.2, $CHCl_3$); UV λ_{max} (log ε) (MeOH) 255 (4.26), 278 sh.; IR ν_{max} cm^{-1}: 1610, 1685, 1735, 2950, 3125, 3155; ^1H and ^{13}C NMR see Tables 1 and 2; DCIMS m/z 330 $[M+H]^+$; EI m/z (rel.int.): 329 (27), 299 (45), 246 (100), 230 (90), 216 (65).

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