The Structure of Sarcomejine: An Application of Long-Range $^{1}H-^{15}N$ Correlation at Natural Abundance

Nikolas Fokialakis,[†] Prokopios Magiatis,[†] Alexios-Leandros Skaltsounis,*[,†] François Tillequin,[‡] and Thierry Sévenet[§]

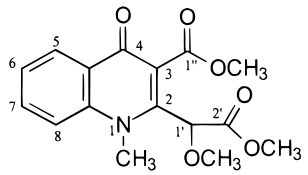
Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens Panepistimiopolis Zografou, GR-15771 Athens, Greece, Laboratoire de Pharmacognosie de l'Université René Descartes, UMR au CNRS No. 8638, Faculté de Pharmacie, 4 Avenue de l'Observatoire, F-75006 Paris, France, and ICSN du CNRS, F-91190 Gif-sur-Yvette, France

Received February 16, 2000

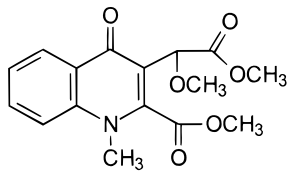
A new 4(1*H*)-quinolinone alkaloid, sarcomejine (**1**), has been isolated from the bark of *Sarcomelicope megistophylla*. Its structure has been elucidated on the basis of MS and NMR data and especially with a long-range $^{1}H-^{15}N$ correlation NMR spectrum at natural abundance.

Sarcomelicope megistophylla Hartley (Rutaceae) is a small- to medium-sized tree, 8−12 m high, easily recognized by its large (up to 34 cm long) pubescent leaves. Hartley described it as endemic to the region of Nouméa, New Caledonia.[1] Recently, we have described the chemical constituents of its leaves[2,3] and the major alkaloids of the bark.[4] In a continuation of our studies of the genus *Sarcomelicope*,[5−12] we report herein the isolation and structure elucidation of a new quinolone alkaloid, from the bark of *S. megistophylla*. Sarcomejine (**1**) was isolated from the dichloromethane extract of the bark. The structure of the novel alkaloid was deduced from ^{1}H and ^{13}C NMR spectral data and by interpretation of its DEPT 135°, COSY 45°, HMQC, and HMBC spectra and from a gradient inverse-detected long-range $^{1}H-^{15}N$ correlation experiment at natural abundance.

Sarcomejine (**1**) was obtained as a yellow amorphous compound, and its molecular formula was determined by HRMS as $C_{16}H_{17}NO_6$. The UV spectrum suggested a quinolone derivative. The ^{1}H NMR spectrum indicated four aromatic protons associated with a nonsubstituted ring A in a 4-quinolone-derived skeleton, three OCH_3 groups (two belonging to methyl ester functional groups and one placed on an sp^3 carbon), one NCH_3 group, and one deshielded aliphatic proton (δ 5.28). The ^{13}C NMR spectrum confirmed the above observations and showed the presence of three carbonyl groups. One (δ 174.7) is included in the quinolone skeleton, and the other two correspond to $COOCH_3$ groups (δ 168.8, 167.6). The sp^3 carbon bearing the methine proton at 5.28 ppm was observed at 78.5 ppm, suggesting that it is an oxygenated carbon attached to an aromatic system. Moreover, three OCH_3 groups and one NCH_3 group were observed at 58.9, 53.3, 52.8, and 35.9 ppm, respectively. Further information on the structure of **1** was obtained from the long-range C−H correlation in the HMBC spectrum (Figure 1). The methine proton at δ 5.28 showed a three-bond correlation with the OCH_3 carbon at δ 58.9 and a two-bond correlation with the carbonyl of the first $COOCH_3$ group at δ 167.6. Additionally, the methine proton showed a correlation with two quaternary aromatic carbons (145.9, 120.5 ppm). One of them (C-2; 145.9 ppm) was correlated with the protons of the NCH_3 group. Given that sarcomejine possessed an *N*-methyl-4-quinolone nucleus



1



2

Figure 1. Sarcomejine (**1**) and the alternative structure **2**.

combined with the above-mentioned HMBC correlations, it could be assigned as either structure **1** or **2**. This problem could not be solved by a NOESY spectrum due to the overlapping of the NMe and the OMe protons of one of the $COOCH_3$ groups (six protons at 3.78 ppm). Therefore, the strong cross-peak observed in the NOESY spectrum (mixing time 700 ms) between the methine proton (5.28 ppm) and a methyl group at 3.78 ppm could not be used as an argument for the unequivocal structure determination of sarcomejine (**1**).

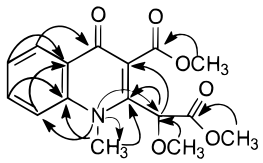
The structure elucidation of this new alkaloid as **1** and discrimination against the alternative structure **2** was provided by long-range $^{1}H-^{15}N$ heteronuclear shift correlation studies. These represent, in the past several years, an area of active research for chemical shift assignments[13−18] and structure elucidation of alkaloids.[19−21] Indeed, the three-bond correlation between ^{15}N-1 (118.3 ppm) and the methine proton (5.28 ppm) (Figure 2) permitted placement of the side chain at C-2 of the 4-quinolone ring and, consequently, placement of the second $COOCH_3$ group at position 3. The experiment was optimized for 5 Hz ^{3}J $^{1}H-^{15}N$ coupling constants.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded in spectroscopic grade MeOH on a Shimadzu-160A spectrophotometer. NMR spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers

* To whom correspondence should be addressed. Tel: +30-1-7274598. Fax: +30-1-7274594. E-mail: skaltsounis@pharm.uoa.gr.
[†] University of Athens.
[‡] Université René Descartes, Paris.
[§] ICSN du CNRS.

10.1021/np000083x CCC: $19.00
Published on Web 06/02/2000



1

Figure 2. Selected HMBC correlations and long-range $^1H-^{15}N$ correlations for sarcomejine (**1**).

[^1H (400 and 200 MHz) and ^{13}C (50 MHz)]; chemical shifts are expressed in parts per million (ppm) downfield from TMS. The $^1H-^1H$ and the $^1H-^{13}C$ NMR experiments were performed using standard Bruker microprograms. For the $^1H-^{15}N$ GHMQC spectrum, data were acquired as 3072 × 400 data points with a total of 290 transients accumulated/t_1 increment. Pulse widths were 8.55 μs for ^1H and 27.7 μs for the ^{15}N at powers of 0 and −3 dB. The F_1 spectral window employed was set from 100 to 400 ppm. Pulsed field gradients, gt1−gt3, had durations of 0.8 ms. Gradient pairs were optimized as 70:30:50 for ^{15}N. EIMS were determined on a HP-6890 and HRMS on a AEI MS-902 spectrometer.

Plant Material. The plant material was collected at Nouméa (New Caledonia) in May 1984. A voucher sample (Pusset-Chauviere 261) is deposited in the herbarium of the Centre ORSTOM at Nouméa, New Caledonia.

Extraction and Isolation. Extraction of alkaloids was as described by Fokialakis et al.[2] The crude alkaloid mixture was chromatographed over a column containing Si gel (Merck 0.04−0.06 mm; flash), using a CH_2Cl_2/MeOH gradient to give seven fractions. Fraction 1 was submitted to flash chromatography on Si gel with CH_2Cl_2/MeOH (99:1) to afford sarcomejine (**1**) (6 mg).

Sarcomejine (1): $[\alpha]^{25}_D$ +3° (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 290 (sh), 341 (3.73), 327 (3.67) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.45 (1H, dd, J = 8.8, 1.5 Hz H-5), 7.71 (1H, td, J = 8.8, 1.5 Hz, H-7), 7.53 (1H, dd, J = 8.8, 1.5 Hz, H-8), 7.42 (1H, td, J = 8.8, 1.5 Hz, H-6), 5.28 (1H, s, H-1′), 3.94 (3H, s, CH_3O-2′), 3.78 (3H, s, $N-CH_3$), 3.78 (3H, s, CH_3O-1″), 3.55 (3H, s, CH_3O-1′); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.7 (C-4), 168.8 (C-1″), 167.6 (C-2′), 145.9 (C-2), 141.5 (C-8a), 133.2 (C-7), 127.0 (C-5), 126.6 (C-4a), 124.6 (C-6), 120.5 (C-3), 115.7 (C-8), 78.5 (C-1′), 58.9 (CH_3O-1′), 53.3 (CH_3O-1″), 52.8 (CH_3O-2′), 35.9 ($N-CH_3$); ^{15}N NMR ($CDCl_3$, 400 MHz) δ 118.3 (N-1); EIMS *m*/*z* 319 (30), 304 (80), 272 (100); HRMS *m*/*z* 319.1052 (calcd for $C_{16}H_{17}O_6N$, 319.1056).

References and Notes

(1) Hartley, T. G. *Bull. Mus. Natl. Hist. Nat., Sec. B: Adansonia* **1986**, *8*, 183−189.
(2) Fokialakis, N.; Mitaku, S.; Mikros, E.; Skaltsounis, A. L.; Tillequin, F.; Sévenet, T. *Phytochemistry* **1999**, *52*, 1745−1748.
(3) Papageorgiou, M.; Fokialakis, N.; Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Sévenet, T. *J. Nat. Prod.* **2000**, *63*, 385−386.
(4) Skaltsounis, A. L.; Seddrati, L.; Tillequin, F.; Koch, M.; Pusset, J.; Sévenet, T. *Nat. Prod. Lett.* **1995**, *5*, 281−287.
(5) Mitaku, S.; Skaltsounis, A.L.; Tillequin, F.; Koch, M.; Pusset, J.; Sévenet, T. *Nat. Prod. Lett.* **1995**, *7*, 219−225.
(6) Mitaku, S.; Pusset, J. *Plant. Méd. Phytothér.* **1988**, *22*, 83−87.
(7) Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pusset, J.; Chauvière, G., *J. Nat. Prod.* **1986**, *49*, 1091−1095.
(8) Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pusset, J. *Ann. Pharm. Fr.* **1989**, *47*, 149−156.
(9) Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pusset J.; Chauvière, G. *Heterocycles* **1987**, *26*, 2057−2063.
(10) Baudouin, G.; Tillequin, F.; Koch, M.; Dau, E. T. H.; Guilhem, J.; Pusset, J.; Chauvière, G. *J. Nat. Prod.* **1985**, *48*, 260−265.
(11) Brum-Bousquet, M.; Tillequin, F.; Koch, M.; Sevenet, T. *Planta Med.* **1985**, *51*, 536−537.
(12) Brum-Bousquet, M.; Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M. *Planta Med.* **1988**, *55*, 191−192.
(13) Martin, G. E.; Crouch, R. C.; Sharaf, M. H. M.; Schiff, P. L. *J. Nat. Prod.* **1996**, *59*, 2−4.
(14) Hadden, C. E.; Martin, G. E. *J. Nat. Prod.* **1998**, *61*, 969−972.
(15) Crouch, R. C.;Martin, G. E. *J. Heterocycl. Chem.* **1995**, *32*, 1665−1669.
(16) Martin, G. E.; Crouch, R. C. *J. Heterocycl. Chem.* **1995**, *32*, 1839−1842.
(17) Martin, G. E.;Crouch, R. C.; Andrews, C. W. *J. Heterocycl. Chem.* **1995**, *32*, 1759−1766.
(18) Martin, G. E. *J. Heterocycl. Chem.* **1997**, *34*, 695−699.
(19) Sharaf, M. H. M.; Schiff, P. L.; Tackie, A. N.; Martin, G. E. *J. Heterocycl.Chem.* **1998**, *35*, 1365−1369.
(20) Koshino, H.; Lee, I. K.; Kim, J. P.; Kim, W. G.; Uzawa, J.; Yoo, I. D. *Tetrahedron Lett.* **1996**, *37*, 4549−4540.
(21) Shin-ya, K.; Kim, J. S.; Furihata, K.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **1997**, *40*, 7079−7082.

NP000083X