HETEROCYCLES, Vol. 63, No. 9, 2004, pp. 2043 - 2050
Received, 11th June, 2004, Accepted, 28th July, 2004, Published online, 30th July, 2004

SARCODIFURINES A AND B, TWO NEW FUROQUINOLINES FROM *SARCOMELICOPE FOLLICULARIS*

Elizabeth Chosson,[a,*] Anne-Emmanuelle Hay,[b] Angele Chiaroni,[c] Anne Favel,[d] François Tillequin,[e] Marc Litaudon,[f] and Elisabeth Seguin[b]

[a] Department of Botany, UFR de Médecine et de Pharmacie, 22 Bd Gambetta F-76183 Rouen cedex 1, France
[b] Department of Pharmacognosy, UFR de Médecine et de Pharmacie, 22 Bd Gambetta F-76183 Rouen cedex 1, France
[c] Department of Crystallography, I.C.S.N., CNRS, Avenue de la Terrasse, F-91198 Gif-sur-Yvette cedex, France
[d] Department of Botany, Cryptogamie et Biologie cellulaire, 27 Bd J. Moulin, 13385 Marseille cedex 5, France
[e] Department of Pharmacognosy, UMR/CNRS. n°8638, Faculté de Pharmacie, 4 Avenue de l'Observatoire, F-75006 Paris, France
[f] I.C.S.N. du CNRS, F-91198 Gif-sur-Yvette cedex, France
E-mail address: Elizabeth.Chosson@univ-rouen.fr

Abstract - Two novel furoquinolines, named sarcodifurine A (**1**) and B (**2**) were isolated from the leaves of *Sarcomelicope follicularis* (Rutaceae). The structures were elucidated on the basis of spectroscopical data, especially 2D-HMBC and HMQC NMR experiments. The relative configurations were unambiguously determined by single-crystal X-Ray diffraction analyses.

The family Rutaceae is a promising source of natural antitumor lead compounds. Indeed, the benzophenanthridine alkaloids nitidine and fagaronine, isolated from several *Zanthoxylum* species exhibit significant antileukemic activity in rodents through DNA topoisomerase I inhibition.[1~3] The indolopyridoquinazolinone evodiamine, found in *Evodia rutaecarpa* displays anti-invasive and anti-metastatic activities.[4,5] The pyranoacridone acronycine, from *Sarcomelicope simplicifolia* (=*Acronychia baueri*) is a potent antitumor agent whose main interest lies in its broad spectrum of activity which includes numerous solid tumors.[6] The corresponding epoxide, isolated from *Sarcomelicope argyrophylla*.[7] led to the development of new antitumor synthetic analogues, including *cis*-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one under current development under the code S23906-1.[8,9] In a continuation of our exploration of the chemical contents of

the *Sarcomelicope* species endemic to New-Caledonia,[10,11] we describe here the structure elucidation of two new difuroquinoline alkaloids isolated from the cyclohexane extract of the leaves of *Sarcomelicope follicularis* Hartley.[12]

Sarcodifurine A (**1**) was obtained as yellow needles from MeOH. The molecular formula was determined as $C_{23}H_{27}NO_5$ through HR-MS spectral analysis (pseudomolecular ion $[M+Na]^+$ at *m/z* 420.1787; calcd: 420.1787). Characteristic absorptions observed at λ_{max} 244, 255(sh), 272 and 320 on the UV spectrum suggested a dihydrofuro[2,3-*b*]quinoline derived basic skeleton.[13,14] The IR spectrum displayed a typical absorption band at 1661 cm^{-1} associated with a conjugated carbonyl group. The ^1H and ^{13}C NMR spectra were assigned by interpretation of COSY, HMQC and HMBC experiments (Table 1). The ^1H NMR spectrum displayed signals typical for two furanic α- and β-protons, two methoxy groups bonded to a sp^2 and a sp^3 carbon (δ_H 4.33 and 2.94, respectively) and a 3-methylbut-2-enyl side chain. Location of the aliphatic methoxy group and the prenyl side chain was ensured by HMBC correlations observed between the signals of C<u>H</u>$_2$ of the prenyl side chain and the quaternary carbon α to the nitrogen on one hand, and between the aliphatic OC<u>H</u>$_3$ group and the sp^3 carbon at δ_C 78.6 (Figure 1) on the other hand. Additional signals, including two 3H-singlets at δ 1.40 and 1.37 and a 3H-doublet at δ 1.11 (*J*=7.2 Hz) coupled with a 1H-quartet at δ 3.04 accounted for a fused 2,2,3-trimethyl-2,3-dihydrofuran ring. X-Ray diffraction analysis permitted us to determine that this latter ring was fused linearly onto the furo[2,3-*b*]quinoline basic skeleton and to establish the relative configuration of sarcodifurine A as (3*R**, 10*S**) (Figure 2).

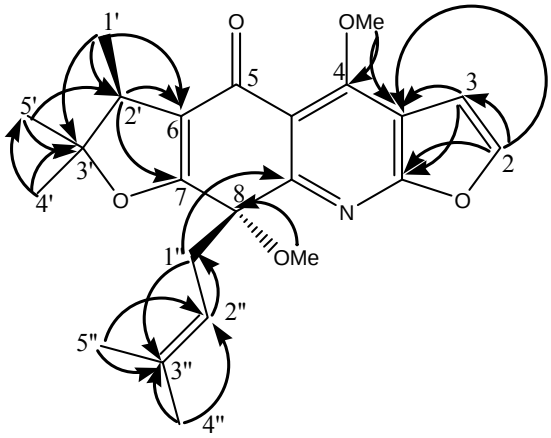


Figure 1. HMBC correlations of **1**

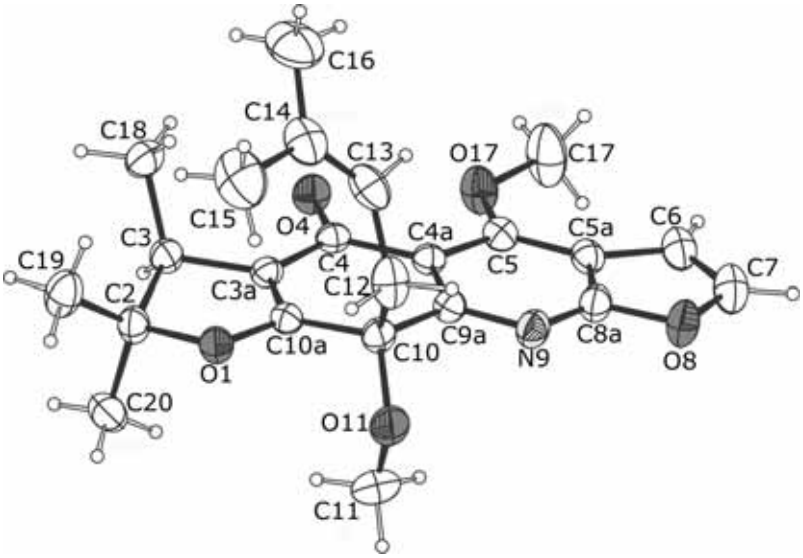


Figure 2. Ortep drawing of **1** showing the atomic numbering scheme. Displacement ellipsoids are shown at the 30% probability level

Sarcodifurine B (**2**) was obtained as yellow needles from MeOH. The molecular formula was determined as $C_{23}H_{27}NO_5$ through HR-MS spectral analysis (pseudomolecular ion $[M+Na]^+$ at *m/z* 420.1771; calcd:

420.1787). The main features of the ^{1}H NMR data were essentially similar with those of **1**, and accounted as previously for two furanic protons, two methoxy groups, a prenyl side chain and a fused trimethyldihydrofuran ring (Table 1). Identical location of the prenyl side chain and aliphatic methoxy groups were deduced from HMBC correlations (Figure 3). Slight differencies observed in the UV spectrum suggested an isomeric angular structure. This hypothesis was confirmed by X-Ray diffraction analysis (Figure 4) which also permitted to establish the relative configuration of sarcodifurine B as (3*R**, 5*R**) (Figure 4).

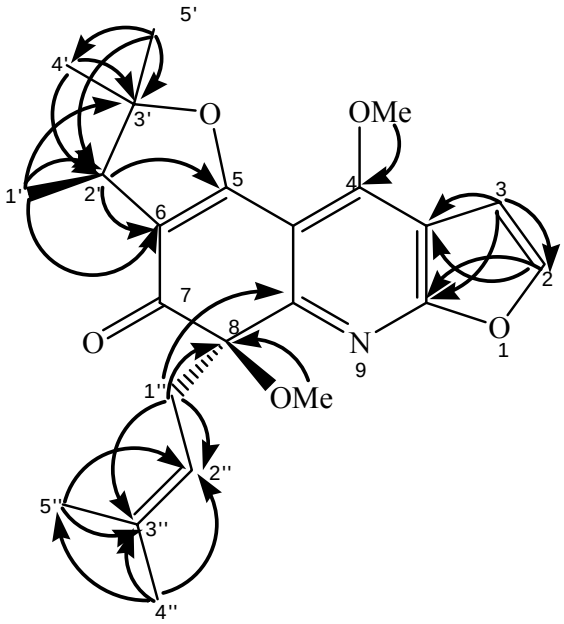


Figure 3. HMBC correlations of **2**

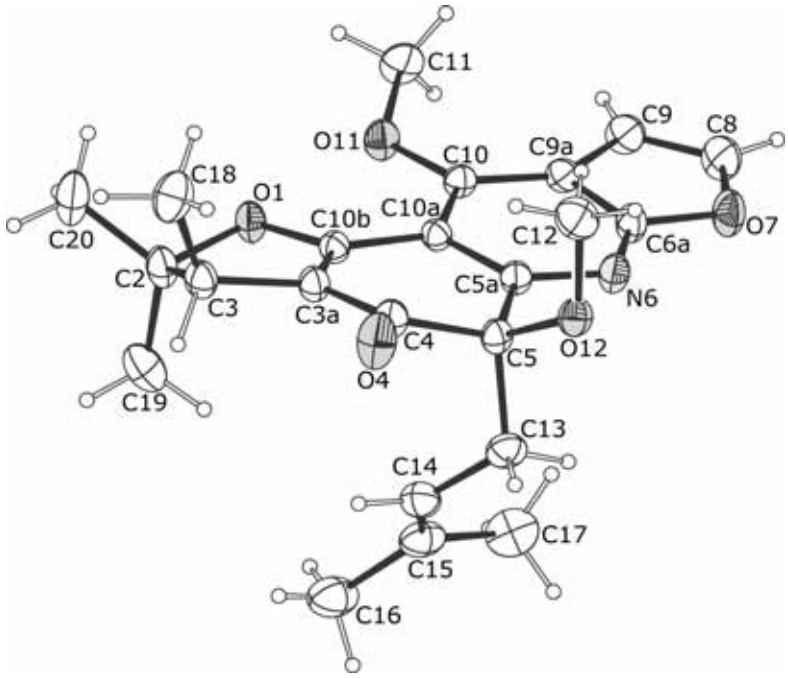


Figure 4. Ortep drawing of **2** showing the atomic numbering scheme. Displacement ellipsoids are shown at the 30% probability level

In both molecules, torsion angles show that the polycyclic core is nearly planar except for the dihydrofuran ring including atom O1, exhibiting an envelope conformation, in which atoms C2 are deviated from the mean plane of the other four atoms, by respectively 0.313(2) Å in **1** and 0.396(2) Å in **2**. In the joined "cyclohexa-2,5-dienone" rings, the carbon atoms C10 in **1** and C5 in **2**, are also slightly deviated from the planarity of the other five atoms, by 0.119(2) and 0.271(2), respectively. The double bonds C6-C7, N9-C8a, C3a-C10a in **1** and the equivalent C8-C9, N6-C6a, C3a-C10b in **2** are well located and of same length. The prenyl chain spread out of the polycyclic systems.

Table 1 ^{1}H and ^{13}C-NMR Spectral Data of **1** and **2**[a)]

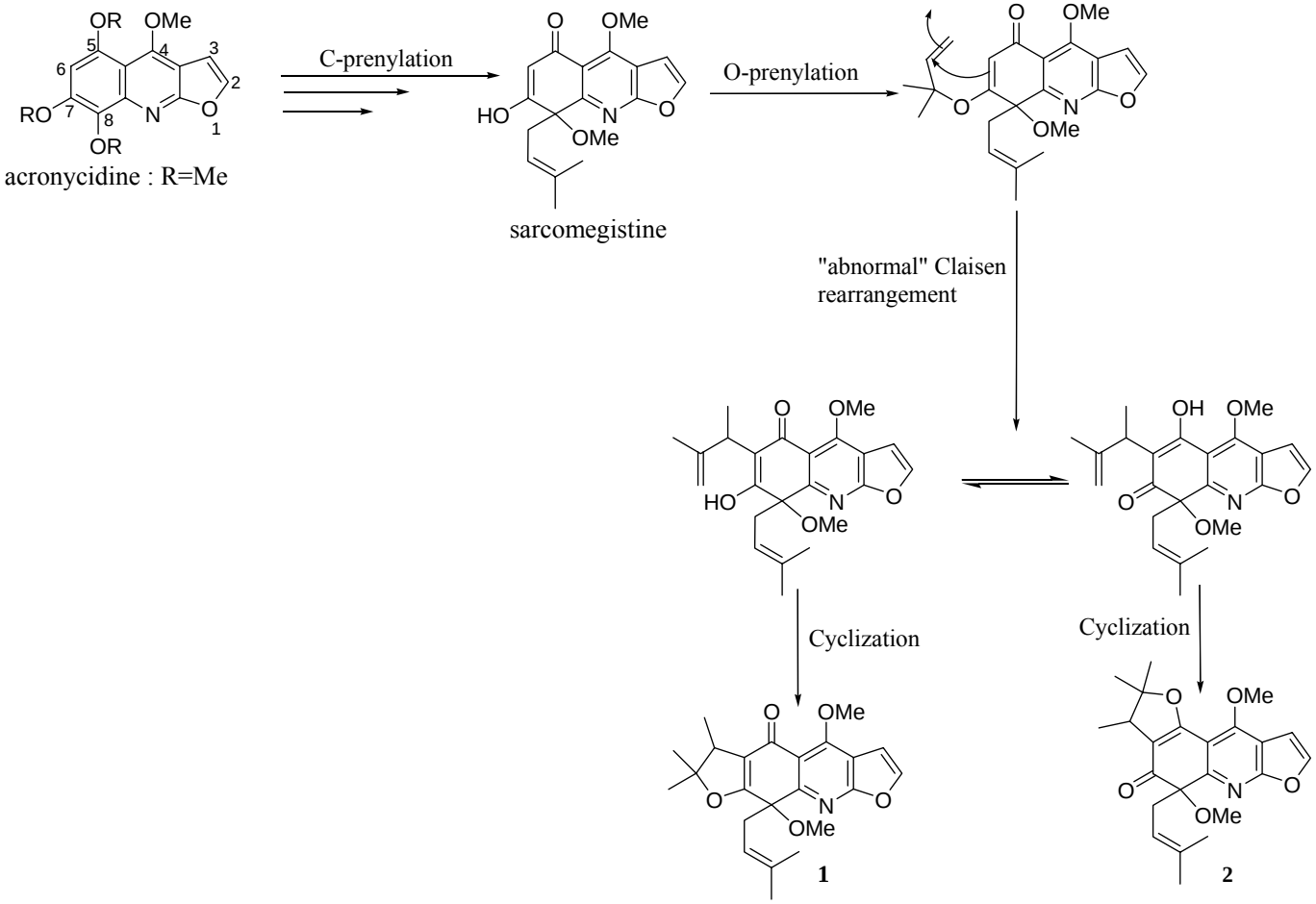
	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2	7.58 d *J*=2.3	143.7	7.59 *J*=2.3	143.9
3	7.04 d *J*=2.3	107.5	6.99 d *J*=2.3	106.4
3a	-	106.9	-	107.2
4	-	162.4	-	159.1
4a	-	116.9	-	109.6
5	-	181.6	-	167.7
6	-	122.7	-	118.9
7	-	168.0	-	193.4
8	-	78.6	-	85.9
8a	-	158.9	-	160.9
9a	-	165.0	-	165.2
1'	1.11 d *J*=7.2	15.0	1.14 d *J*=7.2	15.6
2'	3.04 q *J*=7.2	44.6	2.93 q, *J*=7.2	42.3
3'	-	93.2	-	93.7
4'	1.37[c]	22.4[c]	1.44 s[e]	22.4[e]
5'	1.40[c]	29.3[c]	1.34 s[e]	28.9[e]
1"	3.02 t J=7.5	39.2	2.78 t *J*=7.5	42.7
2"	4.39 t *J*=7.5	116.7	4.55 dd *J*=7.5	116.5
3"	-	136.1	-	136.1
4"	1.40 s	18.4[b]	1.39 s[d]	26.2[d]
5"	1.40 s	26.2[b]	1.26 s[d]	17.9[d]
4-OMe	4.33 s	60.1	4.26 s	60.2
8-OMe	2.94 s	52.9	2.97 s	53.7

a) Chemical shifts are in δ-values from TMS and are followed by multiplicities and *J* values (in Hz), 25°C, in $CDCl_3$ (300 MHz)

b), c), d), e) assignments in the same column with the same superscript are interchangeable

From a biogenetic point of view (Figure 2), sarcodifurines A (**1**) and B (**2**) can be considered to arise from the double prenylation of a 5,7,8-trioxygenated furoquinoline precursor, such as acronycidine isolated from numerous *Sarcomelicope* species.[10] Indeed, alkylation at C_8 by a prenyl unit leads to a dihydrofuroquinoline skeleton such as that previously encountered in sarcomegistine.[13] Further alkylation at O_5 or O_7, followed by "abnormal" Claisen rearrangement and cyclization, can account for the formation

of the additional furan ring present in sarcodifurines A and B.[15] It is interesting to note that a similar fused dihydrofuran ring bearing three methyl substituents is also present in the related furoquinolone spectabiline isolated from several Rutaceae species, including *Ravenia spectabilis*,[16] *Flindersia ifflaiana*,[15] and *Euxylophora paraensis*[17] (scheme 1).



Scheme 1 Biogenetic pathways of sarcodifurines A (**1**) and B (**2**)

Those two new compounds have been tested against different human pathogenic strains of yeasts but did not show any significant activity at 200 µg/mL.

EXPERIMENTAL

General procedures

Melting points were determined on a hot stage Reichert microscope and are uncorrected. Optical rotations were determined in MeOH on a Perkin-Elmer 241 polarimeter. UV spectra were obtained in spectroscopic grade MeOH using a BECKMAN DU 600. IR spectra were recorded on a Nicolet FT-IR 510 spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded at 300 MHz and 75 MHz, respectively with a Bruker Advance 300 spectrometer. Multiple-pulse 2D NMR experiments were performed using standard Bruker microprograms. MS spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers using electrospray ionization (ESI-MS; Vc = 30 V). X-Ray data were obtained with a Nonius-Kappa-CCD area-detector diffractometer, using graphite monochromated Mo Kα radiation, in phi

and omega scans.[21,22] The structures were solved by direct methods using program *SHELXS86*[18]and refined by full-matrix least-squares, based upon unique F^2 with program *SHELXL93*.[19] The hydrogen atoms located in difference Fourier maps were fitted at theoretical positions or treated as riding, and assigned an isotopic displacement parameter equivalent to that of the bonded atom, plus 20%. In the crystal packings, only van der Waals contacts are observed. Crystallographic data (excluding structure factors) for these structures have been depositing with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 240056 and 240057, respectively. Copies can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(01223-336033 or e-mail: deposit@ccdc.cam.ac.uk). Silica gel 60 (Merck, 0.035-0.070 mm) was used for column chromatography, precoated silica gel (Merck, SIL G60 F254, 0.25 mm) was used for analytical TLC.

Plant material

Sarcomelicope follicularis was collected during the flowering phase in January 2001 between Mount Mandjélia and the Tiébo pass. Voucher specimens were deposited in the Herbarium of Botany and Applied Ecology Department, Institut de Recherche pour le Développement, Nouméa, New Caledonia (code NOU) under acquisition number LIT 1297.

Extraction and isolation

1.9 kg of leaves from *S. follicularis* were extracted by cyclohexane (4x2500 mL) during 3 days at rt.The MeOH soluble part (30 g) obtained from this extract was then subjected to silica gel column chromatography using cyclohexane-EtOAc (98 : 2 to 0 : 100, gradient elute) and 100% MeOH as the eluent. This separation gave 490 fractions. Fractions 97-123 (750 mg) were combined and precipitation in cyclohexane gave the alkaloid normelicopicine, identified by comparison with an authentic sample.[20] Recrystallization from MeOH of fractions 268-272 afforded **1** (184 mg), and same operation performed on fractions 320-378 gave **2** (42 mg).

Sarcodifurine A (**1**) : yellow needles (MeOH), mp 155-157°C, $[\alpha]_D^{25}$ -27.0° (c 0.6, MeOH). UV λmax nm : 244, 255(sh), 272 and 320. IR ν_{max} cm^{-1}: 3118, 2979, 2924, 1661, 1643, 1334, 1094, 1011. HRMS *m/z* : $C_{23}H_{27}NO_5$ (M+Na; Calcd for 420.1787 ; found : 420.1787). ^1H and ^{13}C NMR : given in Table 1.

X-Ray structure analysis for **1** : crystal of 0.075x0.175x0.30 mm. Monoclinic system, space group P 2_1, Z = 2. There are two molecules in the unit-cell of parameters: a = 10.091(4), b = 8.957(3), c = 11.732(4) Å, β = 93.69(2)°, V = 1058 $Å^3$, d_c = 1.247 g cm^{-3}, F(000) = 392, λ(Mo Kα) = 0.71073 Å, μ = 0.088 mm^{-1}. 12526 data were collected up to θ = 29.15°. ($-13 \le h \le 13$, $-12 \le k \le 11$, $-16 \le l \le 16$) leading to 5337 unique reflections, of which 3558 were considered as observed having $I \ge 2$ sigma(I).[21,22] Refinement of 270 parameters converged to $R1(F) = 0.0482$ for the observed reflections and $wR2(F^2) = 0.1225$ for all the 5337 data with a goodness-of-fit S factor of 1.017. The residual electron density was found between -0.135 and 0.131 e$Å^{-3}$.

Sarcodifurine B (**2**) : yellow needles (MeOH), mp 166-167°C, intense blue fluorescence at 366 nm. $[\alpha]_D^{25}$ +99.0° (c 0.6, MeOH). UV λmax nm : 258, 267, 276, 354. IR ν_{max} cm^{-1}: 3115, 2923, 2847, 1648, 1597, 1533, 1333, 1128, 781. HRMS *m/z* : $C_{23}H_{27}NO_5$ (M+Na; Calcd for 420.1787 ; found : 420.1771). ^1H and ^{13}C NMR : given in Table 1.

X-Ray structure analysis for **2** : plate crystal of 0.15x0.50x0.55 mm. Orthorhombic system, space group $P2_12_12_1$, Z = 4. There are four molecules in the unit-cell of parameters : a = 10.261(4), b = 10.337(4), c = 19.674(7) Å, V = 2086.8 $Å^3$, d_c = 1.265 g cm^{-3}, F(000) = 848, λ(Mo Kα) = 0.71073 Å, μ = 0.089 mm^{-1}. 21075 data were collected up to θ = 27.46° (-13 ≤ h ≤ 13, -13 ≤ k ≤ 13, -25 ≤ l ≤ 25) leading to 4777 unique reflections, of which 3902 were considered as observed having I ≥ 2 sigma(I). Refinement of 270 parameters converged to $R_1(F)$ = 0.0376 for the observed reflections and $wR_2(F^2)$ = 0.0952 for all the 4777 data with a goodness-of-fit S factor of 1.041. The residual electron density was found between -0.125 and 0.154 $eÅ^{-3}$.

Antifungal activity

Microorganisms, growth conditions, and preparation of fungal inocula

All the yeast strains but one (*Cryptococcus neoformans*) were reference strains : *Candida albicans* ATCC 90029, *C. albicans* ATCC 38248, *C. albicans* Y 0109, *C. tropicalis* IP 1275-81, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 90030, *C. kefyr* Y 0106, *C. krusei* ATCC 6258, *C. lusitaniae* CBS 5094. They were grown on Sabouraud chloramphenicol agar (Pasteur) slants for 48 h at 30°C. Cell suspensions in sterile distilled water were adjusted turbidimetrically at 660 nm to obtain approximately 10^6 CFU/mL.

Susceptibility tests

The antifungal activity of alkaloids was evaluated with the agar dilution method by using solidified (2% agar w/v) yeast nitrogen base (DIFCO). Stock solutions of alkaloids in dimethyl sulfoxide (DMSO) were diluted in the medium, resulting in concentrations of 100 μg/mL and 200 μg/mL. Final concentration of DMSO never exceeded 1%. Yeast nitrogen base agar plates were inoculated in duplicate using a Steer apparatus (multipoint inoculator). They were incubated at 30°C for 48 h. The antifungal agent amphotericin B was included in the assay as positive control. Minimum Fungicidal Concentration (MIC) was defined as the lowest alkaloid concentration showing no visible fungal growth after incubation time.

ACKNOWLEDGEMENTS

We thank S. Versali and K. Fallague for their experimental assistances.

REFERENCES AND NOTES

1. L. Comoë, Y. Carpentier, B. Desoize, and J.-C. Jardillier, *Leukemia Res*., 1988, **12**, 667.
2. L.-K. Wang, R.K. Johnson, and S.M. Hecht, *Chem. Res. Toxicol*., 1993, **6**, 813.
3 T. Ishikawa and H. Ishii, *Heterocycles*, 1999, **50**, 627.

4. M. Ogasawara, T. Matsubara, and H. Suzuki, *Biol. Pharm. Bull*., 2001, **24**, 917
5. M. Ogasawara, T. Matsunaga, S. Takahashi, I. Saiki, and H. Suzuki, *Biol. Pharm. Bull*., 2002, **25**, 1491
6. G.K. Hughes, F.N. Lahey, J.R. Price, and L.J. Webb, *Nature*, 1948, **162**, 223.
7. M. Brum-Bousquet, S. Mitaku, A.-L. Skaltsounis, F. Tillequin, and M. Koch, *Planta Med*., 1988, **54**, 470.
8. N. Costes, H. Le Deit, S. Michel, F. Tillequin, M. Koch, B. Pfeiffer, P. Renard, S. Léonce, N. Guilbaud, L. Kraus-Berthier, A. Pierré, and G. Atassi, *J. Med. Chem*., 2000, **43**, 2395.
9. H.D. Thi Mai, T. Gaslonde, S. Michel, F. Tillequin, M. Koch, J.-B. Bongui, A. Elomri, E. Seguin, B. Pfeiffer, P. Renard, M.-H. David-Cordonnier, W. Laine, C. Bailly, L. Kraus-Berthier, S. Léonce, J.A. Hickam, and A. Pierré, *J. Med. Chem*., 2003, **46**, 3072.
10. F. Tillequin, *Recent Res. Dev. Phytochem*., 1997, **1**, 675.
11. E. Chosson, P. Vérité, A. Blanckaert, E. Seguin, M. Litaudon, and T. Sévenet, *Biochem. Syst. Ecol*., 2003, **31**, 1185.
12. T.G. Hartley, *Bull. Mus. Natn. Hist. Nat., Paris*, 1986,4e sér. 8, 183.
13. A.-L. Skaltsounis, L. Sedrati, F. Tillequin, M. Koch, J. Pusset, and T. Sévenet, *Nat. Prod. Lett*., 1995, **5**, 281.
14. Zs. Rózsa, M. Rábik, K. Szendrei, A. Kálmán, Gy. Argay, I. Pelczer, M. Aynechi, I. Mester, and J. Reisch, *Phytochemistry*, 1986, **25**, 2005.
15. T.R. Chamberlain and M.F. Grundon, *J. Chem. Soc*. (C), 1971, 910.
16. S.K. Talapatra, B.C. Maiti, B. Talapatra, and B.C. Das, *Tetrahedron Lett*., 1969, 4789.
17. L. Jurd and R.Y. Wong, *Aust. J. Chem.*, 1981, **34**, 1625.
18. G.M. Sheldrick, *Acta Cryst. A*, 1990, **46**, 467.
19. G.M. Sheldrick, *SHELXL93*, Program for the refinement of Crystal Structures. University of Göttingen, Germany, 1993.
20. G. Baudouin, F. Tillequin, M. Koch, M.-E. Tran Huu Dau, J. Guilhem, J. Pusset, and G. Chauvière, *J. Nat. Prod*., 1985, **48**, 260.
21. Enraf-Nonius, Kappa-CDD Server software, Enraf-Nonius, Delft, The Netherlands.
22. Z. Otwinowski and W. Minor, *Methods in Enzymology*, 1997, **276**, part A, pp. 307-326.