J|A|C|S
COMMUNICATIONS

Published on Web 05/13/2004

Antifungal Cyclopentenediones from *Piper coruscans*

Xing-Cong Li,*,† Daneel Ferreira,† Melissa R. Jacob,† Qifeng Zhang,† Shabana I. Khan,† Hala N. ElSohly,† Dale G. Nagle,‡ Troy J. Smillie,† Ikhlas A. Khan,†,‡ Larry A. Walker,†,§ and Alice M. Clark*,†,‡,⊥

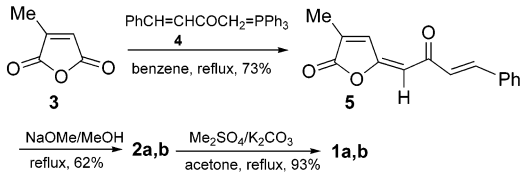
National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, and Department of Pharmacognosy and Department of Pharmacology, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

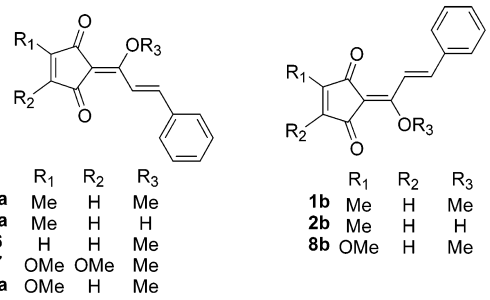
Received April 2, 2004; E-mail: xcli7@olemiss.edu; amc@olemiss.edu

In our search for new prototype antifungal agents, preferably with novel mechanisms of action, from higher plants, the ethanol extract of the whole Peruvian plant, *Piper coruscans* H. B. & K. exhibited significant antifungal activity against *Candida albicans* (IC_{50} < 2 μg/mL). No previous phytochemical or biological studies have been reported on this plant. A subsequent antifungal bioassay-guided fractionation of this extract led to the identification of two new antifungal cyclopentenedione derivatives (**1**, **2**). In this Communication we describe their structure elucidation and antifungal activity.

Coruscanone A (**1**) has a molecular formula of $C_{16}H_{14}O_3$ by high-resolution ESIMS. The ^{1}H NMR spectrum of **1** in benzene-d_6 at room temperature, which was better resolved than in $CDCl_3$, displayed a set of major peaks accompanied by correspondingly less intense peaks with close chemical shifts in a ratio of approximately 5:1. However, various HPLC and GC separations of **1** failed to resolve it, suggesting that compound **1** could be an inseparable mixture of two structurally close isomers with the same molecular weight. Structure elucidation of **1** was thus primarily based on the major peaks in its ^{1}H and ^{13}C NMR spectra. The presence of a styryl moiety was clearly indicated by proton signals at δ 8.36 (d, J = 15.8 Hz), 7.60 (d, J = 15.8 Hz), 7.45 (m), and 7.02 (m) which were correlated with carbon signals at δ 121.1, 142.4, 128.8 (2C), and 129.3 (2C)/130.4, respectively, in its HMQC spectrum. Two carbonyl carbons (δ 195.0 and 191.1) and a relatively deshielded methoxy group (δ_H, 3.99; δ_C, 64.8) were also evident. The remaining two coupled proton signals at δ 6.21 (q, J = 1.5 Hz) and 1.64 (d, J = 1.5 Hz) showed cross-peaks with carbon signals at 140.9 and 10.9, respectively, in the HMQC spectrum. The C−C connectivities were established by HMBC experiments as follows. The proton signal at δ 6.21 correlated with the two carbonyl carbon signals, the methyl signal, and a quaternary carbon signal at δ 109.4, while the methyl signal at δ 1.64 displayed cross-peaks with the carbonyl carbon signal at δ 195.0, the quaternary carbon signal at δ 156.4, and the vinylic carbon signal at δ 140.9. Additionally, the methoxy proton signal at δ 3.99 showed a cross-peak with the quaternary carbon signal δ 168.5, which was correlated with the two vinylic proton signals of the styryl moiety. The above data suggested the structure of compound **1** as 2-(1-methoxy-3-phenyl-2-propenylidene)-4-methylcyclopent-4-ene-1,3-dione. Such a structure explained the coexistence of two geometrical isomers in solution, via photoisomerism, as observed for similar compounds[1] and α-methoxychalcones.[2] Particular attention was then paid to the isomerization process of **1**. When the NMR sample in benzene-d_6 was allowed to sit at room temperature for 24 h, two geometric isomers (**1a**, **1b**) reached a ratio of almost 1:1, indicating similar energies of the two isomers in solution.

Scheme 1. Synthesis of **1** and **2**





	R_1	R_2	R_3
1a	Me	H	Me
2a	Me	H	H
6	H	H	Me
7	OMe	OMe	Me
8a	OMe	H	Me

	R_1	R_2	R_3
1b	Me	H	Me
2b	Me	H	H
8b	OMe	H	Me

Coruscanone B (**2**) displayed ^{1}H and ^{13}C NMR spectra similar to those of **1**. The spectra of **2** differed from those of **1** in that **2** possesses a hydroxyl group on the side chain instead of a methoxy group. This was supported by its high-resolution ESIMS which gave a molecular formula of $C_{15}H_{12}O_3$. In $CDCl_3$, two geometrical isomers appeared in a ratio of approximately 1:1.2 after sitting in the NMR tube at room temperature for 24 h. The slight difference of the ratio of the two isomers resulted in a slight difference of the intensity of the ^{1}H and ^{13}C NMR signals. This facilitated the identification of one set of stronger signals corresponding to the major isomer **2b** from another set of weaker signals corresponding to the minor isomer **2a**. Since within each isomer the carbonyl carbon that forms a hydrogen bond with the allylic hydroxy group should be deshielded,[1b] the key HMBC correlations of the methyl protons and H-4 with carbonyl carbons enabled correlation of the NMR data with respective isomers. This information also facilitated the assignments of the NMR data for two isomers of compound **1**. These assignments, particularly for the carbonyl carbons in **1a**, **1b**, **2a**, and **2b**, are in agreement with those reported for similar compounds which were assigned by conventional 1D NMR experiments, including deuterium isotope effects, C−H coupling constants, and nuclear Overhauser effects.[1b]

Synthesis of coruscanones A and B (**1**, **2**) was achieved by using a method for preparation of similar compounds[3] (Scheme 1). Thus, Wittig condensation of 2-methylmaleic anhydride (**3**) and cinnamoylmethylenetriphenylphosphorane (**4**)[3] in hot benzene afforded

† National Center for Natural Products Research.
‡ Department of Pharmacognosy.
§ Department of Pharmacology.
⊥ Current address: Office of Research, 125 Old Chemistry, The University of Mississippi, University, MS 38677.

10.1021/ja048081c CCC: $27.50

Table 1. Antifungal Activity of **1** and **2** (MIC[a]/MFC[b], μg/mL)

	C. albicans ATCC 90028	*Cr. neoformans* ATCC 90113
1	0.78/1.56	6.25/6.25
2	50/NA[c]	NA/NA
amphotericin B	2.50/5.00	2.50/2.50

[a] Minimum inhibitory concentration. [b] Minimum fungicidal concentration. [c] Not active.

Table 2. Antifungal Activity of **1** against Azole-Susceptible and -Resistant *C. albicans* Strains [IC_{50}/IC_{80}/IC_{95} (μg/mL)][a]

C. albicans	**1**	fluconazole
isolate 1[5a]	0.60/1.00/1.50	1.00/1.50/2.00
isolate 2[5a]	0.90/1.00/1.50	1.00/5.00/10.00
isolate 5[5a]	0.40/0.60/0.70	7.50/10.00/10.00
isolate 8[5a]	0.90/1.00/1.50	15.00/20.00/25.00
isolate 1[5b,c]	0.45/0.60/0.75	0.10/0.20/100.00
isolate 17[5b,c]	0.30/0.90/1.50	40.00/95.00/NA[b]

[a] Patient isolates: isolate 1, azole-susceptible strain; isolates 2, 5, 8, and 17, azole-resistant strains with increasing azole resistance. Growth inhibition concentrations represented by IC_{50}, IC_{80}, and IC_{95} reflect a dose−effect curve. [b] Not active at highest test concentration of 100 μg/mL.

in a regio- and stereoselective manner 4-ylidenebutenolide **5**. Treatment of **5** with NaOMe in MeOH resulted in rearrangement to cylopentendione **2**, which was further methylated with Me_2SO_4/K_2CO_3 in acetone to furnish **1**.

Coruscanones A and B (**1**, **2**) were evaluated using our published bioassay protocols[4] for in vitro antifungal activity. Compound **1** showed potent activity against *Candida albicans* and *Cryptococcus neoformans*, two major opportunistic pathogens associated with AIDS patients (Table 1). Of particular significance is its strong activity against fluconazole-resistant *C. albicans* strains isolated from patients during fluconazole therapy.[5] In the isolates showing up to a 400-fold less susceptibility to fluconazole, **1** retained equivalent activity relative to the susceptible strains (Table 2). It is important to note that *O*-methylation of the enolic hydroxyl group plays a key role in the antifungal activity of coruscanone A when compared to coruscanone B.

Natural cyclopentenediones with structures similar to that of coruscanone A comprise only a few compounds with limited distributions.[6] Recently, a synthetic analogue of this unique class, 2-(1-methoxy-3-phenyl-2-propenylidene)cyclopent-4-ene-1,3-dione (**6**), was identified as a potent inhibitor of human chymase, a potential drug target associated with cardiovascular diseases and chronic inflammation following fibrosis.[7] The synthesis of **6** was inspired by the natural product methyllinderone (**7**),[6a] a moderate inhibitor of the enzyme identified from the screening of a compound library.[7] More recently, methyllucidone (**8a**, **8b**) isolated from *Lindera erythrocarpa* was reported to have moderate antifungal activity against wheat leaf rust caused by *Puccinia recondite*.[8] Although the cyclopent-4-ene- and cyclopentane-1,3-dione structural moieties are present in a number of synthetic compounds with therapeutic applications or potential,[9] the 2-methoxymethylenecyclopent-4-ene-1,3-dione moiety that is responsible for the activity of coruscanone A is an unexplored functional group for antifungal activity against *C. albicans* and *Cr. neoformans*.

It is extremely rare to find a natural product like coruscanone A that exhibits such a high antifungal potency against *C. albicans*. Coruscanone A shows similarity to some extent to 1,4-benzoquinone compounds whose biological action is often linked to electron-transfer rates and redox potentials.[10] It is likely, however, that coruscanone A functions through a unique antifungal mechanism. Taking into account its antifungal potency, certain selectivity, acceptable cytotoxicity (comparable to amphotericin B, refer to Supporting Information), and its ready access by synthesis, coruscanone A may serve as a template for a new class of antifungal agents for the treatment of life-threatening disseminated candidiasis.[11] Investigation of its mechanism of action using a genomic profiling approach[12] and preparation of a series of derivatives for structure−activity relationship studies are underway in our laboratory.

Acknowledgment. We thank Dr. Ted White and Dr. Spencer Redding for kindly providing the fluconazole-resistant *C. albicans* strains, Dr. Charles D. Hufford for critically reviewing the manuscript, Dr. D. Chuck Dunbar and Mr. Frank M. Wiggers for obtaining some spectroscopic data, and Dr. Ameeta Agarwal, Ms. Sharon Sanders, and Ms. Belynda Smiley for technical assistance. This work was supported by the NIH, NIAID, Division of AIDS, Grant No. AI 27094, and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009.

Supporting Information Available: Experimental details and ^{1}H NMR spectra of key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Forsén, S.; Merényi, F.; Nilsson, M. *Acta Chem. Scand.* **1964**, *18*, 1208. (b) Ng, S.; Lee, H.-H.; Bennett, G. J. *Magn. Reson. Chem.* **1990**, *28*, 337.
(2) Ferreira, D.; Roux, D. G. *J. Chem, Soc. Perkin Trans. I* **1977**, 134.
(3) Clemo, N. G.; Gedge, D. R.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1448.
(4) (a) Li, X.-C.; ElSohly, H. N.; Nimrod, A. C.; Clark, A. M. *J. Nat. Prod.* **1999**, *62*, 767. (b) Li, X.-C.; Jacob, M. A.; ElSohly, H. N.; Nagle, D. G.; Smillie, T. J.; Walker, L. A. Clark, A. M. *J. Nat. Prod.* **2003**, *69*, 1132.
(5) (a) Marr, K. A., Lyons, C. N.; Rustad, T.; Bowden, R. A.; White, T. C. *Antimicrob. Agents Chemother*. **1998**, *42*, 2584. (b) White T. C. *Antimicrob. Agents Chemother*. **1997**, *41*, 1482. (c) Pfaller, M. A.; Rhine-Chalberg, J.; Redding, S. W.; Smith, J.; Farinacci, G.; Fothergill, A. W.; Rinaldi, M. G. *J. Clin. Microbiol.* **1994**, *32*, 59.
(6) (a) Kiang, H. H.; Sim, K. Y. *J. Chem. Soc.* **1962**, 4338. (b) Lee, H. H. *Tetrahedron Lett.* **1968**, 4243. (c) Liu, S. Y.; Ogihara, Y. *Yakugaku Zasshi* **1975**, *95*, 114. (d) Leong, Y.-W.; Harrison, L. J.; Bennett, G. J.; Kadir, A. A.; Connolly, J. D. *Phytochemistry* **1998**, *47*, 891.
(7) Aoyama, Y.; Konoike, T.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1695.
(8) Choi, Y. H.; Kwon, S. Y.; Kim, J. H.; Beak, N. I.; Choi, G. J.; Cho, K. Y.; Lee, B. M. *Han'guk Nonghua Hakhoechi* **2003**, *46*, 151.
(9) (a) Grohe, K.; Frohberger, P. E.; Scheinpflug, H. DE 2248819, 1974. (b) Inayama, S.; Mamoto, K.; Shibata, T.; Hirose, T. *J. Med. Chem.* **1976**, *19*, 433. (c) Kawada, H.; Hayashi, S.; Kasugai, A.; Shigematsu, T. JP 52079022, 1977. (d) Simonov, V. V.; Anishchenko, A. F.; Popova, E. N.; Dunaeva, T. P.; Gazizov, R. T.; Simonov, V. D. DE 2804271, 1978. (e) Iwataki, I.; Shibuya, M.; Nakada, A.; Mizuno, M. JP 53101336, 1978. (f) Kobayashi, E.; Koyama, N.; Kato, I. PCT Int. Appl., WO 9904777, 1999. (g) Shestak, P.; Novikov, V. L.; Stekhova, S. I.; Gorshkova, I. A. *Pharm. Chem. J.* **1999**, *33*, 18. (h) Hori, H.; Nagasawa, H.; Ishibashi, M.; Uto, Y.; Hirata, A.; Saijo, K.; Ohkura, K.; Kirk, K. L.; Uehara, Y. *Bioorg. Med. Chem.* **2002**, *10*, 3257.
(10) (a) Voet, D.; Voet, J. B.; Pratt, C. W. *Fundamentals of Biochemistry*; Wiley: New York. 1999. (b) Adams, R. D.; Miao, S. *J. Am. Chem. Soc.* **2004**, *126*, 5056.
(11) (a) Odds, F. C. *Int. J. Stand. AIDS* **1992**, *3*, 157. (b) Coleman, D. C.; Bennett, D. E.; Sullivan, D. J.; Gallagher, P. J.; Henman, M. C.; Shanley, D. B.; Russell, R. J. *Crit. Rev. Microbiol.* **1993**, *19*, 61.
(12) Agarwal, A. K.; Rogers, P. D.; Baerson, S. R.; Jacob, M. R.; Barker, K.; Cleary, J. D.; Walker, L. A.; Nagle, D. G., Clark, A. M. *J. Biol. Chem.* **2003**, *278*, 34998.

JA048081C