Communications to the Editor

Guanacastepene, a Fungal-Derived Diterpene Antibiotic with a New Carbon Skeleton

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Roughly a quarter of all known biologically active natural products have come from fungi,[1,2] and the overwhelming majority of fungal natural products have come from filamentous fungi.[3] Since the number of fungal species is conservatively estimated at 1.5 million of which only 5000−7000 are currently in culture collections and available for study,[3−5] fungi should remain a rewarding source of new natural products. Endophytic fungi, the fungi including filamentous fungi that live in the intracellular spaces of vascular plants, are thought to be the largest pool of fungal diversity.[4,6,7] As part of a project exploring the endophytic fungi of Costa Rica, fungal extracts were screened for antibiotic activity against drug-resistant strains of *Staphylococcus aureus* and *Enterococcus faecalis*. Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecalis* (VREF) are two of the most problematic drug-resistant pathogens, and their increasing frequency has become a major concern.[8,9] New antibiotics—presumably different from currently employed antibiotics—are needed to control these and other drug-resistant pathogens. The extract of a fungus isolated from the branch of a *Daphnopsis americana* tree harvested from the Guanacaste Conservation Area in Costa Rica has excellent activity against both MRSA and VREF. In this paper we report the isolation and characterization by NMR and X-ray crystallography of guanacastepene (**1**), a new MRSA and VREF active diterpene antibiotic.

Hexane extracts from fungal cultures grown in potato dextrose broth (14−21 days) were initially partitioned by C-18 flash column chromatography using a CH_3CN:H_2O step gradient (40:60, 50:50, 70:30, and 100:0). The major active component was pooled from fractions that contained a single UV active compound (TLC: C-18 $R_f = 0.45$, 70:30, CH_3CN:H_2O, silica gel $R_f = 0.70$, 95:5, $CHCl_3$:MeOH) to give an essentially pure compound that was given the trivial name guanacastepene.[10] Guanacastepene was then further purified to homogeneity by semiprep reversed phase ODP-50 HPLC (CH_3CN:H_2O, 33:67).

Table 1. ^{13}C and ^1H NMR Data for the Two Conformers of Guanacastepene Present at −50 °C

	major		minor	
	^{13}C[a]	^1H[b,c]	^{13}C[a]	^1H[b,c]
1	149.4		149.3	
2	133.3	7.43 (s)	131.7	7.47 (s)
3	158.2		161.5	
4	140.1		138.6	
5	62.7	4.64 (m)	59.5	4.52 (m)
OH		4.58 (d, 5.0)		4.48 (d, 4.5)
6	27.8	1.70 (2H)	27.8	1.70 (2H)
7[d]	35.5	1.43, 1.85	29.9	1.21, 2.13
8	38.8		41.9	
9[d]	38.5	1.60, 2.18	35.6	1.36, 1.59
10[d]	35.5	2.00, 2.08	32.0	1.40, 2.11
11	46.5		47.7	
12	54.9	1.90	50.5	2.00
13	74.7	5.45 (d, 7.5)	73.5	5.52 (d, 8.5)
14	200.3		201.1	
15	193.3	9.96 (s)	192.6	9.73 (s)
16	24.6	1.06 (3H, s)	26.9	1.04 (3H, s)
17	20.2	1.25 (3H, s)	24.6	1.13 (3H, s)
18	25.7	1.91	26.0	1.91
19	23.4	0.86 (3H, d, 6.0)	23.3	0.85 (3H, d, 6.5)
20	23.6	1.08 (3H, d, 6.0)	23.7	1.08 (3H, d, 6.0)
21	170.2		170.3	
22	21.0	2.11 (3H, s)	21.0	2.10 (3H, s)

[a] ^{13}C spectrum was recorded at 100 MHz in acetone-d_6 (referenced at 29.9 ppm). [b] ^1H spectrum was recorded at 500 MHz in acetone-d_6 (referenced at 2.05 ppm). [c] (Multiplicity, J in Hz) chemical shifts without multiplicity were determined by $^1H-^{13}C$ HMQC. [d] Assignments are interchangeable.

Room-temperature ^{13}C NMR spectrum showed only 13 signals although the molecular formula, $C_{22}H_{30}O_5$, determined by HR-FABMS, indicated the presence of 22 carbons.[11] Only a limited number of functional groups: one aldehyde, one acetate, an additional carbonyl, two conjugated double bonds, one alcohol and one isobutyl group, with limited connectivity to each other were apparent in room temperature 2-D NMR experiments. The presence of two conformers in a dynamic equilibrium at room temperature was suggested by low-temperature ^1H and ^{13}C NMR. At −50 °C each of the signals present at room temperature resolved into two independent signals, and additional signals appeared above the baseline (Table 1). The two conformers that were resolved at −50 °C exist in a ratio of approximately 2 to 3.

Because of the complexity of guanacastepene's NMR spectra, we elected to continue the structure determination using single-

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(1) Henkel, T.; Brunne, R. M.; Muller, H.; Reichel, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 643−647.
(2) Pearce, C. *Adv. Appl. Microbiol.* **1997**, *44*, 1−80.
(3) Dreyfuss, M. M.; Chapela, I. H. In *The Discovery of Natural Products with Therapeutic Potential*; Gullo, V. P., Ed.; Butterworth-Heinemann: Boston, MA, 1994.
(4) Hawksworth, D. L. *Mycol. Res.* **1991**, *95*, 641−655.
(5) Hawskworth, D. L.; Kirsop, B. E. *Living Resources for Biotechnology, Filamentous Fungi*; Cambridge University Press: Cambridge, MA, 1988.
(6) Pimm, S. L.; Russell, G. J.; Gittleman, J. L.; Brooks, T. M. *Science* **1995**, *269*, 347−350.
(7) Zhang, B.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M. T.; Pelaez, F.; Ruby, C.; Kendall, R. L.; Mao, X.; Griffin, P.; Calaycay, J.; Zierath, J. R.; Heck, J. V.; Smith, R. G.; Moller, D. E. *Science* **1999**, *284*, 974−977.
(8) Neu, H. C. *Science* **1992**, *257*, 1064−1073.
(9) Swartz, M. N. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2420−2427.
(10) Attempts to identify the producing fungus have been unsuccessful and thus this compound was named after the Guanacaste Conservation Area.

(11) HRMS-FAB (m/z): $[M + H^+]^+$ calcd for $C_{22}H_{31}O_5$ 375.2171, found 375.2157; $[\alpha]^{25}_D$ +61.2° (c 1.0, acetone); UV_{max} = 230, 295 nm (CH_3CN); IR (NaCl, thin film) ν_{max} 3446, 1748, 1370, 1220, 1018 cm^{-1}; ^{13}C NMR at 25 °C in acetone-d_6 200.2, 193.0, 169.9, 149.2, 139.3, 132.4, 74.4, 47.0, 27.7, 25.8, 23.5, 23.2, 20.8; ^1H NMR at 25 °C in acetone-d_6 9.91 (s), 7.45 (d, 1.5), 5.48 (d, 8.5), 4.63 (m), 3.97 (OH, s), 2.08 (3H, s), 1.99 (m), 1.90 (m), 1.79 (m), 1.63 (m), 1.40 (m), 1.27 (3H, s), 1.12 (3H, d, 8.0), 1.09 (3H, s), 0.93 (3H, d, 7.5).

(12) Guanacastepene crystallized at −20 °C from acetone, and the X-ray crystal structure used a 0.3 × 0.15 × 0.08 mm crystal. Guanacastepene crystallized in space group $P2_12_12_1$ with unit cell dimensions a = 8.2220(1) Å, b = 10.923(2) Å, c = 23.337(3) Å. The final refined conventional R-factor for 2164 unique reflections with $|F_o| > 4\sigma(F_o)$ was 7.5%. Crystallographic data for guanacastepene have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax: +44-(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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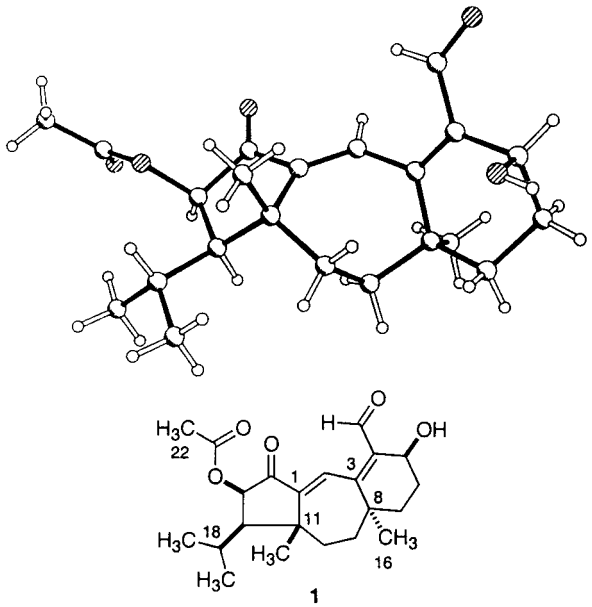

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Figure 1. Computer-generated perspective drawing and chemical drawing of guanacastepene (**1**). The absolute configuration shown is arbitrary.

crystal X-ray diffraction techniques.[12] The X-ray crystal structure (Figure 1), which defines only the relative configuration, reveals that guanacastepene is a roughly rectangular tricyclic [5−7−6] diterpene with one highly oxidized long edge and a hydrophobic opposite edge. The tricyclic ring system of guanacastepene is essentially planar with the exception of C9, which protrudes out of the plane to accommodate the central cycloheptane ring.

The missing room-temperature ^{13}C NMR signals (C-3, 5, 7, 8, 9, 10, 12, 16, and 17) are clustered around the central cycloheptane ring, suggesting that the NMR anomalies could be the result of flexibility in the C9−C10 region of this ring. Using the MM2 force field[13] we identified two gauche butane-like conformers around the C9−C10 bond that differ by 0.14 kcal/mol and are separated by a 15 kcal/mol energy barrier (Figure 2). Thus, the broad and missing NMR signals are likely the result of having both conformers populated and slowly interconverting on the NMR time scale at room temperature.

The guanacastane skeleton,[14] which has not been previously reported, is related to the marine-derived dolastane[15,16] family of diterpenes by a 1,2-methyl shift across the C1−C11 ring juncture and to the neodolabellane family by the closure of the macrocycle to give the tricyclic [5−7−6] ring system (Figure 3). The guanacastane skeleton has been proposed as a biosynthetic intermediate of the trichoaurantiane skeleton.[17] However, while more than 140 dolabellanes[18] and a much smaller number of neodolabellanes and tricyclic dolastanes have been isolated, the guanacastane skeleton has not been described.

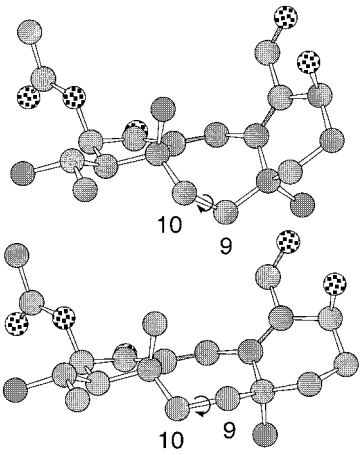

Figure 2. The two lowest energy gauche butane-like conformers calculated for the dihedral angle defined by C-8, -9, -10, and -11. The top model depicts the orientation of the C9−C10 bond that was observed in the X-ray crystal structure and is only 0.14 kcal/mol higher in energy than the conformation depicted by the lower model. At room temperature the interconversion between these two conformers likely masks many of the NMR signals.

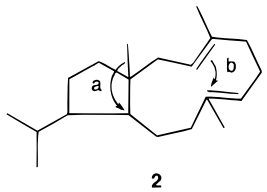

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Figure 3. The neodolabellane, dolastane, and guanacastane carbon skeletons can be derived from the dolabellane skeleton (**2**) by the biosynthetic transformations a, b, or a+b, respectively. Neodolabellane requires the 1,2-methyl shift across the C1−C11 ring juncture (a), neodolabellane the closure of the macrocycle (b) and guanacastane both the 1,2-methyl shift and the closure of the macrocycle (a+b).

In agar diffusion assays run on bacterial lawns pure guanacastepene shows antibiotic activity against methicillin-sensitive and -resistant *S. aureus* and vancomycin-resistant *E. faecalis*. Against MRSA 100 μg of guanacastepene or vancomycin produce 11 and 17 mm zones of growth inhibition, respectively. While vancomycin is ineffective against VREF, guanacastepene produced a 9 mm zone of growth inhibition. Further modes of action studies are now being conducted and will be reported in due course.

Fungi have traditionally been a very rewarding source of biologically active natural products. Endophytic fungi, which can be easily isolated in large numbers from vascular plants, now provide the opportunity to expand even further the chemical and structural diversity already observed among fungal secondary metabolites.[2,4,7]

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Supporting Information Available: NMR spectra and archival X-ray data for guanacastepene (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) *CS Chem3D Pro*, version 3.5; CambridgeSoft Corporation: Cambridge, MA, 1997.

(14) We propose that the name guanacastane be used for this family of diterpenes.

(15) Pettit, G. R.; Ode, R. H.; Herald, C. L.; Von Dreele, R. B.; Michel, C. *J. Am. Chem. Soc.* **1976**, *98*, 4677−4678.

(16) Rao, C. B.; Pullaiaih, R. K.; Surapaneni, R. K.; Sullivan, B. W.; Albizati, K. F.; Faulkner, D. J.; Cun-heng, H.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 2736−2742.

(17) Invernizzi, A. G.; Vidari, G.; Vita-Finzi, P. *Tetrahedron Lett.* **1995**, *36*, 1905−1908.

(18) Rodriquez, A. D.; Gonzalez, E.; Ramirez, C. *Tetrahedron* **1998**, *54*, 11683−11729.