Human Cytochrome P450 Liability Studies of *trans*-Dihydronarciclasine: A Readily Available, Potent, and Selective Cancer Cell Growth Inhibitor

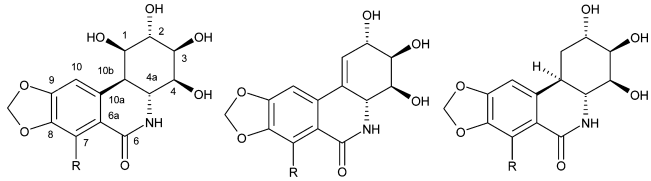
James McNulty,*[,†] Amol Thorat,[†] Nesrin Vurgun,[†] Jerald J. Nair,[†] Emilija Makaji,[‡] Denis J. Crankshaw,[‡] Alison C. Holloway,[‡] and Siyaram Pandey[§]

Department of Chemistry and Chemical Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada, L8S 4M1, Department of Obstetrics and Gynecology, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5, and Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario, Canada, N9B 3P4

Received September 21, 2010

The cytochrome P45O activities of the naturally occurring Amaryllidaceae alkaloid narciclasine (**3**), isolated from *Narcissus pseudonarcissus,* and synthetic derivative *trans*-dihydronarciclasine (**5**) are reported. While narciclasine was found to possess potent inhibitory activity to human CYP3A4, its dihydro analogue was inactive. This study revealed that the C1−C10b double bond is required for inhibition of this crucial metabolizing enzyme. Compound **5** also demonstrated no inhibition of the related human cytochromes CYP19 and CYP1A1. This study elevates the status of *trans*-dihydronarciclasine (**5**) as a highly privileged, readily available molecule, with potent and selective anticancer activity.

The Amaryllidaceae alkaloids have attracted significant attention by virtue of their diverse structures[1] coupled with important biological activities that have been documented for members of this family. The lycorane derivatives pancratistatin[2] (**1**) and narciclasine (**3**), in particular, have attracted considerable interest over the last two decades due to the potent, selective anticancer activity demonstrated, as well as their unknown mechanism of action.[2d,3] Recent studies showed that pancratistatin induces apoptosis, or programmed cell death, selectively in cancer cells, with minimal effect on normal cells, and that the mitochondria in cancerous cells are a site of action.[3,4b] Pancratistatin was shown to have a greater specificity than etoposide (VP-16) or paclitaxel in selectively inducing apoptosis. Other recent studies have shown that narciclasine disrupts organization of the actin skeleton in cancer cells at low (30−90 nM) concentrations.[3e] Narciclasine (**3**) has also been shown to increase survival in preclinical models of human glioblastoma multiforme and clearly has much potential.[3f]

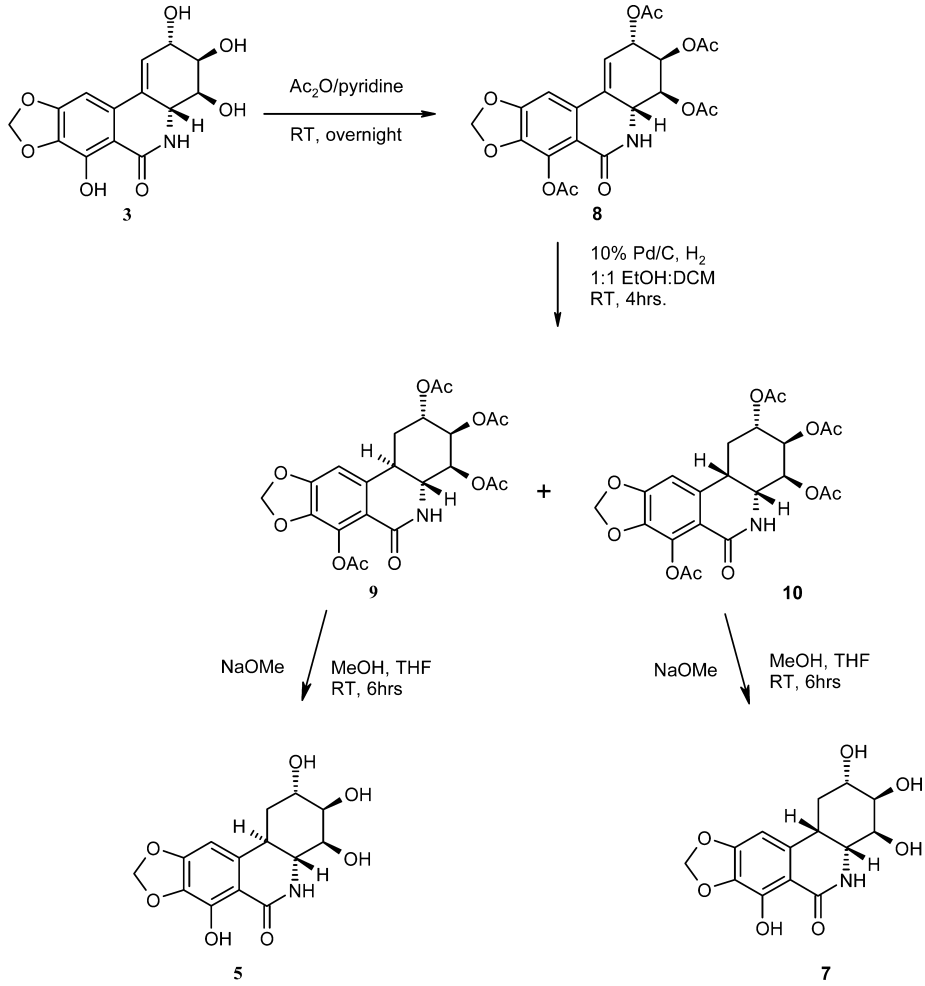


Three major issues have been of concern in the development of a clinically relevant schedule involving pancratistatin or a derivative. The first issue is its low aqueous solubility (53 μg/mL), a problem that has been tackled through the synthesis of phosphate derivatives[4] including conversion of natural pancratistatin to the biologically active 7-phosphate pro-drug.[4b] A second issue is the low natural abundance of pancratistatin[2a,b] and hence access to a reliable supply. Two solutions to this problem are the semisynthetic conversion of the readily available derivative narciclasine (**3**) to pancratistatin (**1**) (10 steps, 3.6% overall yield)[5] or total synthesis.[6] These two issues have served to refocus attention on the relatively abundant alkaloid (**3**) over the past few years.[3e,f,7,9] Narciclasine along with pancratistatin and the naturally occurring derivatives 7-deoxypancratistatin (**2**), 7-deoxynarciclasine (**4**), *trans*-dihydronarciclasine (**5**) (also known as 1-deoxypancratistatin), and 7-deoxy-*trans*-dihydronarciclasine (**6**) all demonstrate potent anticancer activity. These derivatives share the same profile and hence elusive mechanism of action as determined through COMPARE pattern-recognition analysis.[2d] While *trans*-dihydronarciclasine (**5**) has potent anticancer activity,[2d,8] *cis*-dihydronarciclasine (10b epimer of **5**) was inactive,[2d] demonstrating the well-known fine subtleties of this anticancer pharmacophore. Narciclasine (**3**) is available in relatively large quantities from many of the Amaryllidaceae, including common varieties of daffodil (*Narcissus pseudonarcissus*).[7c] The synthesis and biological assessment of a large number of narciclasine derivatives, including water-soluble pro-drugs, was recently reported by Kiss and co-workers[3e] and further semisynthetic interconversions by Pettit et al.[8] The third issue facing the development of a viable clinical derivative in the series is the determination of their potential toxicity, particularly their liability in terms of interaction with human cytochrome P450s. Surprisingly, this issue has been overlooked, and our group recently documented the selective interaction of certain of these alkaloids with human cytochromes, in particular CYP3A4.[9] The CYP3A subfamily comprises about 30% of the total liver cytochrome P450 enzyme pool in humans, and the isoenzyme CYP3A4 accounts for approximately 60% of drugs metabolized.[10c] Many drug development programs have failed or are dose-compromised due to interactions with this most predominant enzyme.[10] Inhibition of CYP3A4 can lead to drug accumulation, contribute significantly to negative drug−drug interactions, and may lead to hepatic toxicity. In our recent study,[9] narciclasine (**3**) was shown to be a potent inhibitor of human CYP3A4 ($K_i = 0.63$ μM), raising serious concerns for the future development of a narciclasine derivative. This study also revealed considerable selectivity, most importantly showing that pancratistatin was inactive to CYP3A4.[9] These results immediately draw attention to the double bond in narciclasine as a likely site of interaction with CYP3A4. In the present work, we report the isolation and conversion of natural narciclasine (**3**) to the known anticancer derivative *trans*-dihydronarciclasine (**5**) (1-deoxypancratistatin)[2d,11] and demonstrate that this derivative, like native pancratistatin, exhibits no inhibition of CYP3A4 and other human cytochrome P450s.

* To whom correspondence should be addressed. E-mail: jmcnult@mcmaster.ca.
† Department of Chemistry and Chemical Biology, McMaster University.
‡ Department of Obstetrics and Gynecology, McMaster University.
§ University of Windsor.

10.1021/np100657w
Published on Web 11/24/2010

Scheme 1. Synthesis of *cis*- and *trans*-Dihydronarciclasine



Results and Discussion

Although *trans*-dihydronarciclasine (**5**) is a natural product,[2d] it is not readily available, and we elected to pursue a semisynthetic approach. Narciclasine (**3**) was isolated from freshly harvested bulbs of *N. pseudonarcissus* according to a literature protocol.[12]

The tetraacetate derivative (**8**) was prepared under standard acetylation conditions as outlined in Scheme 1. Stereoselective hydrogenation of the double bond proved capricious and was highly dependent on the solvent employed. Pettit and co-workers studied this hydrogenation reaction (1 atm H_2, 25 °C) in a variety of solvents and found that the highest stereoselectivity favoring the *trans*-dihydro diastereomer (**9**) was obtained in a 1:1 ratio of EtOH/CH_2Cl_2 (*trans*:*cis* 65:25).[8] The diastereomer ratio also varied considerably upon the nature of the Pd catalyst employed. In our hands, initial hydrogenation of (**8**) in EtOH/CH_2Cl_2, 1:1, over 10% Pd/C (4 h, rt) favored formation of the *cis*-diastereomer (**10**), which was isolated in 60% yield. In this reaction, hydrogen was allowed to bubble through the reaction medium at room temperature, which, it was subsequently realized, resulted in partial loss of volatile CH_2Cl_2, altering the facial selectivity of the hydrogenation. In separate experiments, the catalyst was first purged with hydrogen in the presence of the tetraacetate (**8**), followed by addition of solvent (EtOH/CH_2Cl_2, 1:1). This protocol resulted in formation of the *trans*-dihydro diastereomer (**9**) (isolated in 50% yield), confirming the important role of CH_2Cl_2 for selective hydrogenation. Removal of the acetate protecting groups independently from (**9**) and (**10**) then provided a sample of *trans*-dihydronarciclasine (**5**), identical to that reported, and *cis*-dihydronarciclasine (**7**).

Although *cis*-dihydronarciclasine has been reported several times in the literature,[2d,8,14a] no X-ray data are available, and the direct correlation of the structure with its NMR data proved difficult. Since isomerization of the olefinic double bond is known to occur in this series under hydrogenation conditions,[14a] other isomers are possible. We were able to crystallize a sample of compound (**7**) and characterize the molecule via single-crystal X-ray structural analysis for the first time, confirming the structure and absolute configuration as shown (Figure 1).

The interaction of *trans*-dihydronarciclasine (**5**) with human CYP3A4 was investigated under standard conditions, via kinetic monitoring of the conversion of 7-benzyloxyquinoline to 7-hydroxyquinoline by fluorometric measurement of emission at 538

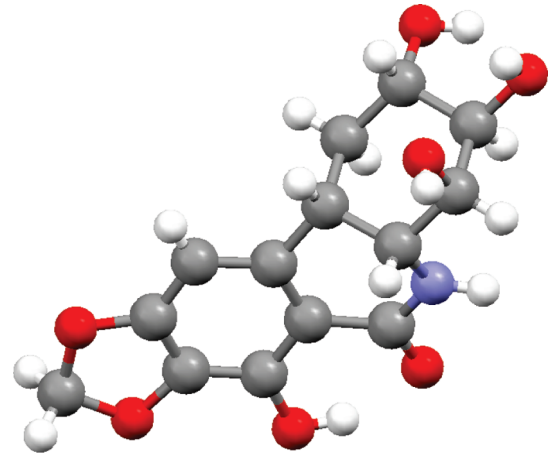


Figure 1. X-ray structure of *cis*-dihydronarciclasine **7**.

nm after excitation at 410 nm, using ketoconazole as a positive control. While **3** proved to be a potent inhibitor of CYP3A4 (K_i = 0.63 μM), **5** was found to be inactive, similar to the data described for pancratistatin.[9] Dihydronarciclasine (**5**) also proved inactive to the related human cytochromes P450s CYP1A1 and CYP19 at 10 μM. This study confirms the critical role of the C1−C10b double bond in derivatives such as narciclasine for CYP3A4 interaction. These P450 liability studies also now confer a privileged status to *trans*-dihydronarciclasine (**5**),[2d] a compound that has attracted a recent flurry of synthetic activity,[15] toward the development of a readily available, potent, selective anticancer agent.

Acknowledgment. Financial support of this work by the Lotte and John Hecht Memorial Foundation, NSERC, and McMaster University is gratefully acknowledged. We thank Dr. D. Hughes for high-field NMR and Dr. J. Britten for the X-ray structural elucidation.

Supporting Information Available: Isolation of native narciclasine, synthetic procedures, ^{1}H and ^{13}C NMR spectra of all compounds, and crystallographic data for compound **7** (tables of selected bond lengths and angles) are available free of charge via the Internet at http://pubs.acs.org. Full details have been deposited at the Cambridge Crystallographic Data Centre under deposition number CCDC 786216 (www.ccdc.cam.ac.uk).

References and Notes

(1) (a) Cook, J. W., Loudon, J. D. Alkaloids of the Amaryllidaceae. In *The Alkaloids*; Manske, R. H. F., Holmes, H. L., Eds.; Academic Press: New York, 1952; Vol. II, pp 331−352. (b) Wildman, W. C. The Amaryllidaceae Alkaloids. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic Press: New York, 1968; Vol. XI, pp 307−405. (c) Martin, S. F. The Amaryllidaceae Alklaoids. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1987; Vol. 30, pp 251−376. (d) Jin, Z. *Nat. Prod. Rep.* **2007**, *24*, 886–905. (e) Evidente, A.; Kireev, A. S.; Jenkins, A. R.; Romero, A. E.; Steelant, W. F. A.; van Slambrouck, S.; Kornienko, A. *Planta Med.* **2009**, *75*, 501–507.

(2) (a) Pettit, G. R.; Gaddamidi, V.; Cragg, G. M.; Herald, D. L.; Sagawa, Y. *J. Chem. Soc., Chem. Commun.* **1984**, 1693–1694. (b) Pettit, G. R.; Gaddamidi, V.; Cragg, G. M. *J. Nat. Prod.* **1984**, *47*, 1018–1020. (c) Pettit, G. R.; Gaddamidi, V.; Herald, D. L.; Singh, S. B.; Cragg, G. M.; Schmidt, J. M.; Boettner, F. E.; Williams, M.; Sagawa, Y. *J. Nat. Prod.* **1986**, *49*, 995–1002. (d) Pettit, G. R.; Pettit, G. R., III; Backhaus, R. A.; Boyd, M. R.; Meerow, A. W. *J. Nat. Prod.* **1993**, *56*, 1682–1687.

(3) (a) McLachlan, A. M.; Kekre, N.; McNulty, J.; Pandey, S. *Apoptosis* **2005**, *10*, 619–630. (b) Kekre, N.; Griffin, C.; McNulty, J.; Pandey, S. *Cancer Chemother. Pharmacol.* **2005**, *56*, 29–38. (c) Pandey, S.; Kekre, N.; Naderi, J.; McNulty, J. *Artifi. Cells, Blood. Sub. Biotech.* **2005**, *33*, 279–295. (d) Siedlakowski, P.; McLachlan-Burgess, A.; Griffin, C.; Tirumalai, S.; McNulty, J.; Pandey, S. *Cancer Biol. Ther.* **2008**, *7*, 376–384. (e) Ingrassia, L.; Lefranc, F.; Dewelle, J.; Pottier, L.; Mathieu, V.; Spiegl-Kreinecker, S.; Sauvage, S.; El Yazidi, M.; Dehoux, M.; Berger, W.; van Quaquebeke, E.; Kiss, R. *J. Med. Chem.* **2009**, *52*, 1100–1114. (f) Lefranc, F.; Sauvage, S.; Van Goietsnoven, G.; Megalizzi, V.; Lamoral-Theys, D.; Debeir, O.; Spiegl-Kreinecker, S.; Berger, W.; Mathieu, V.; Decaestecker, C.; Kiss, R. *Mol. Cancer Ther.* **2009**, *8*, 1739–1750.

(4) (a) Pettit, G. R.; Freeman, S.; Simpson, M. J.; Thomson, M. A.; Boyd, M. R.; Williams, M. D.; Pettit, G. R., III; Doubek, D. L. *Anti-Cancer Drug Des.* **1995**, *10*, 243–250. (b) Shnyder, S. D.; Cooper, P. A.; Millington, N. J.; Gill, J. H.; Bibby, M. C. *J. Nat. Prod.* **2008**, *71*, 321–324.

(5) Pettit, G. R.; Melody, N.; Herald, D. L. *J. Org. Chem.* **2001**, *66*, 2583–2587.

(6) (a) Rinner, U.; Hudlicky, T. *Synlett.* **2005**, 365–387. (b) Chapleur, Y.; Chretien, F.; Ibn-Ahmed, S.; Khaldi, M. *Curr. Org. Synth.* **2006**, *3*, 169–185. (c) Manpadi, M.; Kornienko, A. *Org. Prep. Proc. Int.* **2008**, *40*, 107–161.

(7) (a) Ceriotti, G. *Nature* **1967**, *213*, 595. (b) Savona, G.; Piozzi, F.; Marino, M. L. *J. Chem. Soc., Chem. Commun.* **1970**, 1006. (c) Kornienko, A.; Evidente, A. *Chem. Rev.* **2008**, *108*, 1982–2014.

(8) Pettit, G. R.; Ducki, S.; Eastham, S. A.; Melody, N. *J. Nat. Prod.* **2009**, *72*, 1279–1282.

(9) McNulty, J.; Nair, J. J.; Singh, M.; Crankshaw, D. J.; Holloway, A. C.; Bastida, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3233–3237.

(10) (a) Shaik, S.; Cohen, S.; Wang, Y.; Chen, H.; Kumar, D.; Thiel, W. *Chem. Rev.* **2010**, *110*, 949–1017. (b) Arimoto, R.; Prasad, M. A.; Gifford, E. M. J. *Biomol. Screen.* **2005**, *10*, 197–205. (c) Nelson, D. R.; Zeldin, D. C.; Hoffman, S. M. G.; Maltais, L. J.; Wain, H. W.; Nebert, D. W. *Pharmacogenetics* **2004**, *14*, 1. (d) Cesar, T. B.; Manthey, J. A.; Myung, K. *J. Nat. Prod.* **2009**, *72*, 1702–1704.

(11) Pettit, G. R.; Ducki, S.; Eastham, S. A.; Melody, N. *J. Nat. Prod.* **2009**, *72*, 1279–1282.

(12) Dumont, P.; Ingrassia, L.; Rouzeau, S.; Ribaucour, F.; Thomas, S.; Roland, I.; Darro, F.; Lefranc, F.; Kiss, R. *Neoplasia* **2007**, *9*, 766–776.

(13) Pettit, G. R.; Cragg, G. M.; Singh, S. B.; Duke, J. A.; Doubek, D. L. *J. Nat. Prod* **1990**, *53*, 176–178.

(14) For prior reports on *cis*-dihydronarciclasine see refs 2d and 8 above and: (a) Mondon, A.; Krohn, K. *Chem. Ber.* **1975**, *108*, 445–463. (b) Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kefauver, D. F.; Pettit, G. R.; Groszek, G.; Hollingshead, M.; Kirsi, J. J.; Shannon, W. M.; Schubert, E. M.; DaRe, J.; Ugarkar, B.; Ussery, M. A.; Phelan, M. J. *J. Nat. Prod.* **1992**, *55*, 1569–1581.

(15) For recent syntheses of *trans*-dihydronarciclasine see: (a) Shin, I. J.; Choi, E. S.; Cho, C. G. *Angew. Chem., Int. Ed.* **2007**, *46*, 2303–2305. (b) Jana, C. K.; Studer, *Chem.—Eur. J.* **2008**, *14*, 6326–6328. (c) Cho, Y. S.; Cho, C. G. *Tetrahedron* **2008**, *64*, 2172–2177.

NP100657W