Biomimetic Synthesis and Structure Elucidation of Rubicordifolin, a Cytotoxic Natural Product from *Rubia cordifolia*

Jean-Philip Lumb and Dirk Trauner*

Center for New Directions in Organic Synthesis, Department of Chemistry, University of California, Berkeley, California 94720-1460

Received December 19, 2004; E-mail: trauner@cchem.berkeley.edu

Cycloadditions are unmatched in their ability to generate molecular diversity and stereochemical complexity. Hence, it is no surprise that they are widely found in biosynthetic pathways.[1] While some naturally occurring cycloadditions have been shown to require enzyme catalysis,[2] many proceed spontaneously, often yielding complex racemic products.

Racemic natural products with intricate structures have indeed been found in certain plants of the genus *Rubiaceae* (Figure 1).[3] Rubioncolin A (**1**) and rubioncolin B (**2**) were isolated from *Rubia oncotricha*.[4] The latter compound was also found in the Chinese medical plant *Rubia cordifolia*.[5] An unnamed natural product, **3**, was isolated from this source as well, whose relative configuration was not reported. Compound **3** showed significant cytotoxic activity both in vitro and in vivo, inhibiting the growth of sarcoma ascites in mice at low concentrations.[5]

We now report a concise synthesis of **3** resulting somewhat serendipitously from our systematic investigations of the *Rubiaceae* natural products. In the course of our studies, the structure of **3** has been fully elucidated, prompting us to name this intriguing compound "rubicordifolin".

Biosynthetic and retrosynthetic analysis suggests that the dimeric natural products shown in Figure 1 ultimately stem from prenylated naphthoquinone **4** through a series of oxidations, electrocyclizations, and cycloadditions (Scheme 1).

Naphthoquinone **4** is a known natural product previously isolated from various *Rubiaceae*.[6] Oxidation with concomitant allylic transposition would afford the reactive vinyl quinone **5** as a hypothetical biosynthetic intermediate. Acid-catalyzed or photochemical isomerization of this material would yield naphthofuran **6**.[7] A [4 + 2] cycloaddition involving **5** and **6** would then give rubioncolin A (**1**).[8] Similar, yet more elaborate, biosynthetic schemes can be devised to account for the formation of **2** and **3**.

Inspired by this biosynthetic hypothesis, we set out to synthesize vinyl quinone **4** and to study its isomerization to naphthofuran **5** and subsequent dimerization (Scheme 2). Conjugate addition of a vinyl cuprate derived from vinyl stannane **7**[9] to 2-carbomethoxy-naphthoquinone **8**,[10] followed by tautomerization, gave vinyl naphthohydroquinone **9**. Oxidation with aqueous cerium ammonium nitrate not only restored the quinone moiety but also resulted in cleavage of the silyl ether to afford **5**.

Upon standing at room temperature, the unstable vinyl quinone **5** underwent conversion into naphthofuran **6** and furomollugin (**11**). This reaction presumably proceeds through cation **10**, which undergoes either deprotonation to afford **6** or *retro*-Friedel−Crafts hydroxyalkylation to yield **11**.[11] Furomollugin (**11**) is a biologically active natural product that has been isolated from several members of the *Rubiaceae* family.[12]

With vinyl quinone **5** and naphthofuran **6** in hand, we studied their dimerization to afford rubioncolin A (**1**). While these studies have so far not afforded **1**, they have resulted in a concise synthesis
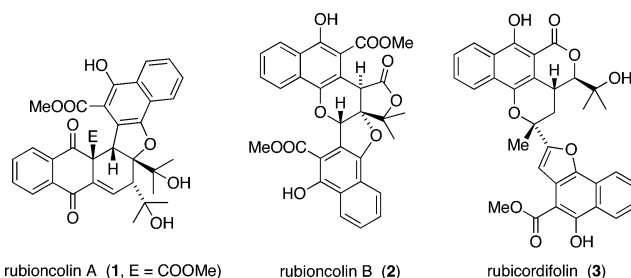
**Figure 1.** Complex natural products isolated from *Rubiaceae*.

rubioncolin A (**1**, E = COOMe)   rubioncolin B (**2**)   rubicordifolin (**3**)

Scheme 1. Biosynthetic Hypothesis
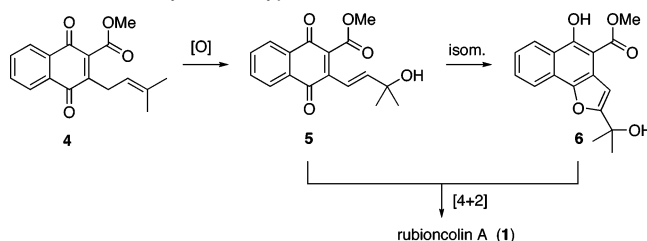
Scheme 2. Synthesis of Building Blocks **5**, **6**, and Furomollugin (**11**)[a]
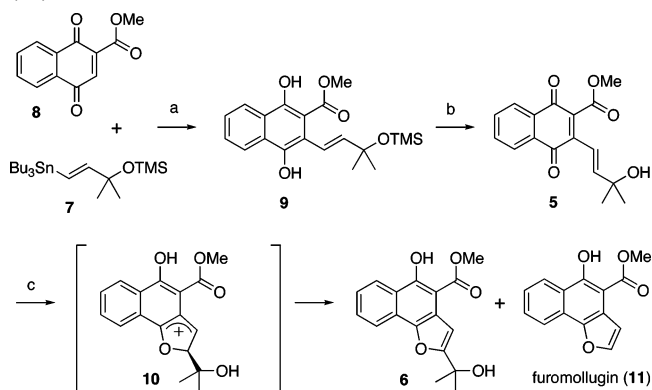
[a] Reagents and conditions: (a) **7**, *n*-BuLi, CuCN, THF, → **8**, → NH₄Cl/NH₄OH (67%); (b) CAN, MeCN, H₂O (99%); (c) THF, rt (23% of **6**; 24% of **11**).

of rubicordifolin (**3**) (Scheme 3). Attempting to promote the dimerization by transiently tethering the tertiary alcohol moieties of **5** and **6** with phenylboronic acid,[13] we isolated **3** in low yield, along with elimination product **12**.

Mechanistically, this result can be interpreted as follows (Scheme 4). Phenylboronic acid-mediated cyclization of **5** affords **13**, whose intramolecular demethylation gives *ortho*-quinone methide **14**.[14] Simultaneously, dehydration of naphthofuran **6** occurs. The resultant electron-rich alkene **12** then undergoes regioselective hetero-Diels−Alder reaction with *ortho*-quinone methide **14**, followed by hydrolysis, to afford the natural product **3**. The stereochemical

Scheme 3. Synthesis of Rubicordifolin (**3**)
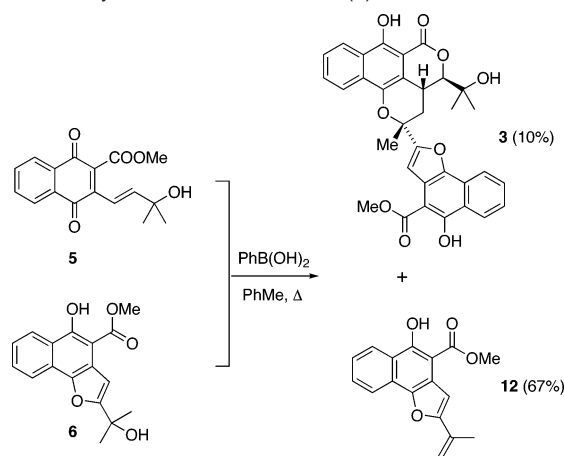
Scheme 4. Mechanistic Interpretation
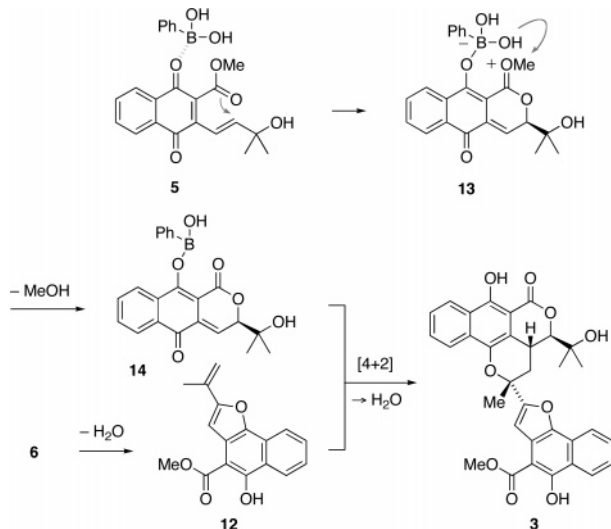
outcome of this cycloaddition is compatible with an *endo*-transition state, whose induced diastereoselection is governed by the bulky hydroxyisopropyl group.

Synthetic rubicordifolin (**3**) is identical in all respects (^{1}H NMR, ^{13}C NMR, IR, UV, and MS) with the previously unnamed cytotoxic natural product isolated from *Rubia cordifolia*.[5,15] Its relative stereochemistry was elucidated by detailed nOe measurements (see Supporting Information).

Since both heterodiene **14** and dienophile **12** ultimately stem from vinyl naphthoquinone **5**, it was tempting to investigate the direct conversion of **5** into **3**. Indeed, under carefully optimized conditions, **5** underwent cyclization and dimerization to afford rubicordifolin *in 45% yield*. In this case, furomollugin (**11**) was formed as the major byproduct. Given the multitude of individual steps and associated rate constants that need to be orchestrated for this reaction to occur, the yield of 45% is remarkably high. Note that the vinyl substituent of **5** has to engage in cyclizations with both proximate carbonyl groups at a comparable rate to afford the dimerization partners **14** and **12**.

In summary, we have achieved a concise, biomimetic synthesis of rubicordifolin and fully established the structure of the natural product.[16] The synthesis yields hundreds of milligrams of the biologically active natural product and could be easily modified to produce analogues of rubicordifolin for further biological testing.

Scheme 5. Direct Synthesis of Rubicordifolin (**3**) and Furomollugin (**11**)
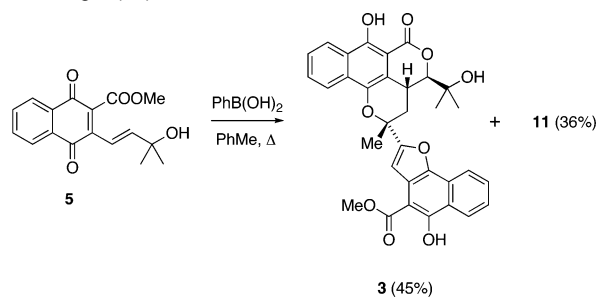
Synthetic studies on these analogues, as well as rubioncolins A (**1**) and B (**2**), and other naphthoquinone derivatives isolated from *Rubiaceae* are well underway in our laboratories and will be reported in due course.

Note Added after ASAP Publication: In the version published on the Internet February 12, 2005, naphthofuran was mistakenly identified as compound **4** in Scheme 4 and in the fourth line of the last paragraph on the first page. The final version published February 15, 2005 and the print version correctly identify it as compound **6**.

Supporting Information Available: Synthetic procedures and spectroscopic data for compounds **3**, **5**, **6**, **7**, **9**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Stocking, E. M.; Williams, R. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 3078. (b) Malerich, J. P.; Trauner, D. *J. Am. Chem. Soc.* **2003**, *125*, 9554.
(2) Ichihara, A.; Oikawa, H. In *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Meth-Kohn, O., Eds.; Elsevier: Amsterdam, 1999; Vol. 1, pp 307−408.
(3) Chauhan, S.; Singh, R. *Chem. Biodiversity* **2004**, *1*, 1241.
(4) Qiao, Y. F.; Takeya, K.; Itokawa, H.; Iitaka, Y. *Chem. Pharm. Bull.* **1990**, *38*, 2896.
(5) (a) Itokawa, H.; Ibraheim, Z. Z.; Qiao, Y. F.; Takeya, K. *Chem. Pharm. Bull.* **1993**, *41*, 1869. (b) Compound **3** was also isolated from *Rubia alata*: Quao, Y.-F., Ph.D. Thesis, Tokyo College of Pharmacy, 1991.
(6) Heide, L.; Leistner, E. *J. Chem. Soc., Chem. Commun.* **1981**, 334.
(7) For the conversion of vinyl quinones to benzofurans, see: (a) Iwamoto, H.; Takuwa, A.; Hamada, K.; Fujiwara, R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 575. (b) Taing, M.; Moore, H. W. *J. Org. Chem.* **1996**, *61*, 329.
(8) For vinyl quinones in Diels−Alder reactions, see: Noland, W.; Kedrowski, B. *J. Org. Chem.* **1999**, *64*, 596.
(9) Vinyl stannane **7** was prepared by silyl protection of the corresponding, known alcohol: Zhang, H. X.; Guibe, F.; Balavoine, G. *J. Org. Chem.* **1990**, *55*, 1857.
(10) Barker, D.; Brimble, M. A.; Do, P.; Turner, P. *Tetrahedron* **2003**, *59*, 2441.
(11) For an example of a *retro*-Friedel−Crafts reaction, see: Rousseau, G.; Robin, S. *Tetrahedron Lett.* **2000**, *41*, 8881
(12) (a) Ho, L. K.; Don, M. J.; Chen, H. C.; Yeh, S. F.; Chen, J. M. *J. Nat. Prod.* **1996**, *59*, 330−333. (b) Itokawa, H.; Qiao, Y. F.; Takeya, K. *Phytochemistry* **1991**, *30*, 637.
(13) Nicolaou, K. C.; Liu, J. J.; Hwang, C. K.; Dai, W. M.; Guy, R. K. *J. Chem. Soc., Chem. Commun.* **1992**, 1118.
(14) For a review on the chemistry of *ortho*-quinone methides, see: Van de Water, R. W.; Pettus, T. R. R. *Tetrahedron* **2002**, *58*, 5367.
(15) Unfortunately, an authentic sample of natural **3** was not available to us.
(16) For other examples of dimeric phenolic natural products obtained through biomimetic synthesis, see: (a) Chapman, O. L.; Engel, M. R.; Springer, J. P.; Clardy, J. C. *J. Am. Chem. Soc.* **1971**, *93*, 6696. (b) Nicolaou, K. C.; Simonsen, K. B.; Vassilikogiannakis, G.; Baran, P. S.; Vidali, V. P.; Pitsinos, E. N.; Couladouros, E. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 444. (c) Barnes-Seeman, D.; Corey, E. J. *Org. Lett.* **1999**, *1*, 1503. (d) Krohn, K. In *Progress in Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G. W., Eds.; Springer: Vienna and New York, 2003; Vol. 85, pp 1−49.

JA042375G