

Total Synthesis and Absolute Configuration of Otteliones A and B, Novel and Potent Antitumor Agents from a Freshwater Plant

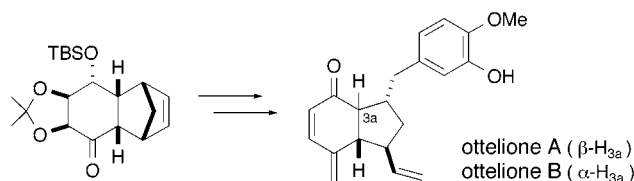
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ABSTRACT



The first enantioselective total synthesis of otteliones A and B, biologically important and structurally novel natural products, has been successfully achieved. This total synthesis fully confirms the absolute configuration of these natural products.

Otteliones A (**1**) and B (**2**) (Figure 1) were isolated by Ayyad and Hoye et al. in 1998 from the freshwater plant *Ottelia alismoides* collected in the Nile Delta, Egypt.^{1,2} These substances were found to exhibit prominent biological properties such as antitubercular³ and antitumor^{1,2} activities. Remarkably, these small-molecule natural products displayed quite potent cytotoxicity at nM–pM levels of IC₅₀ values against a panel of 60 human tumor cell lines at the National Cancer Institute in the United States.^{1,4} It has also been reported that **1** inhibits tubulin polymerization into microtubules (IC₅₀ = 1.2 μ M) similarly to well-known colchicine, vincristine, and vinblastine.⁵ Consequently, otteliones are now serving as new

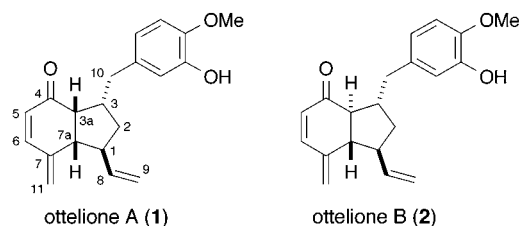


Figure 1. Structures of otteliones A (**1**) and B (**2**).

leads for the development of cancer chemotherapeutic agents. However, further biological studies are severely limited by the scarcity of these natural products.⁶

Structurally, otteliones A and B possess a novel bicyclic hydrindane skeleton with four contiguous asymmetric car-

(5) Combeau, C.; Provost, J.; Lancelin, F.; Tournoux, Y.; Prod'homme, F.; Herman, F.; Lavelle, F.; Leboul, J.; Vuilhorgne, M. *Mol. Pharm.* **2000**, *57*, 553.

(6) Otteliones A (**1**) and B (**2**) had been obtained in 0.0009% yield (dry weight) from the plant *Ottelia alismoides*; see ref 1.

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(1) Ayyad, S.-E. N.; Judd, A. S.; Shier, W. T.; Hoye, T. R. *J. Org. Chem.* **1998**, *63*, 8102.

(2) Incidentally, ottelione A (**1**) was identical with the compound named RPR112378 previously reported in the patent literature by a research group at Rhône-Poulenc Rorer (now Aventis); see: Leboul, J.; Provost, J. French Patent WO96/00205 1996; *Chem. Abstr.* **1996**, *124*, 242296.

(3) Li, H.; Qu, X.; Shi, Y.; Guo, L.; Yuan, Z. *Zhongguo Zhongyao Zazhi (Chin. J. Chin. Mater. Med.)* **1995**, *20*, 115, 128.

(4) Ottelione A (**1**) has been also shown to inhibit doxorubicin-resistant leukemia cells (P388/DOX) with an IC₅₀ value of 1 ng/mL (3 nM); see ref 1.

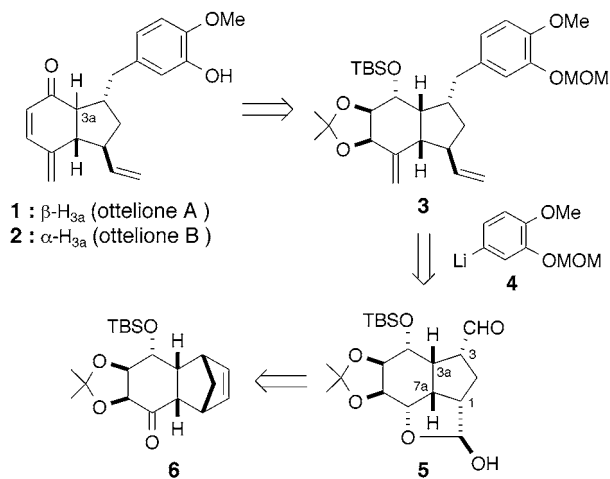
bons, in which the rare and highly sensitive 4-methylene-2-cyclohexenone substructure is a particularly characteristic feature. While the relative configurations of otteliones A and B were deduced by high-field NMR spectroscopy,^{1,5} their absolute configurations remain uncertain. The remarkable biological properties, unique structural features, and limited availability from natural resources, as well as the need to confirm the absolute configuration, have made the otteliones exceptionally intriguing and timely targets for total synthesis.

A number of synthetic approaches toward otteliones A and B have been reported to date.⁷ The first elegant total synthesis of racemic (\pm)-otteliones A (**1**) and B (**2**) was recently achieved by Mehta et al.⁸ and provided proof of their relative stereochemistry.

Recently, we embarked on a project directed at the total synthesis of optically active otteliones A and B, as well as their analogues, with the aim of exploring their structure–activity relationships. In this communication, we report the first enantioselective total synthesis of otteliones A (**1**) and B (**2**), which proves their absolute configuration to be as depicted in Figure 1.⁹

The key element of our synthetic plan for **1** and **2** outlined in Scheme 1 is our utilization of the highly and appropriately

Scheme 1. Synthetic Plan for Otteliones A (**1**) and B (**2**)



functionalized intermediate **5**, which contains not only the requisite bicyclic hydrindane core structure having the correct stereogenic centers at C3, C3a, and C7a but also the desirable functionalities for elaboration of **1** and **2**. Since the C1 formyl

(7) (a) Clive, D. L. J.; Fletcher, S. P. *Chem. Commun.* **2002**, 1940. (b) Mehta, G.; Islam, K. *Org. Lett.* **2002**, *4*, 2881. (c) Mehta, G.; Islam, K. *Synlett* **2000**, 1473. (d) Trembleau, L.; Patiny, L.; Ghosez, L. *Tetrahedron Lett.* **2000**, *41*, 6377. (e) Mehta, G.; Reddy, D. S. *Chem. Commun.* **1999**, 2193.

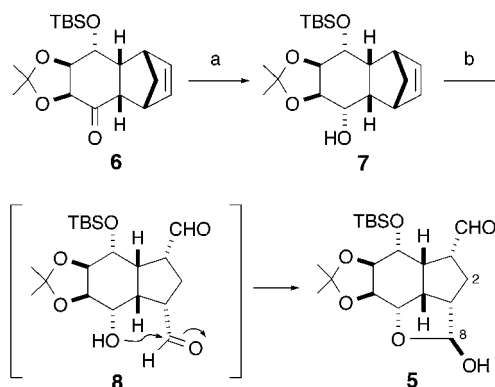
(8) Mehta, G.; Islam, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 2396.

(9) After submission of this manuscript, we learned that Mehta and Islam had completed an enantioselective total synthesis of both enantiomers of otteliones A (**1**) and B (**2**) and elucidated the absolute configurations of naturally occurring **1** and **2**; see: Mehta, G.; Islam, K. *Tetrahedron Lett.* **2003**, *44*, 6733. The absolute configuration assignments independently achieved by the Mehta group are compatible with our own results described in this communication.

group of **5** would be masked as an internal hemiacetal moiety, the advanced key intermediate **3** would be elaborated through the coupling reaction of **5** with aryllithium **4** followed by epimerization at C1 and further functionalization. The intermediate **3** would be converted to the target molecules **1** and **2** by sequential functional group manipulation and deprotection, or vice versa. Intermediate **5** could in turn be accessed from the known tricyclic compound **6**,¹⁰ which has previously been prepared in an enantiomerically pure form in our laboratory.

At first, as shown in Scheme 2, we pursued the synthesis of the key intermediate **5** starting with the tricyclic compound

Scheme 2. Synthesis of Key Intermediate **5**^a



^a Reagents and conditions: (a) NaBH₄, THF–H₂O, 0 °C, 87%. (b) OsO₄, NaIO₄, *t*-BuOH–THF–H₂O, 0 °C → rt, 62%.

6.¹⁰ Thus, sodium borohydride reduction of **6** provided the expected product **7** in 87% yield. Subsequent oxidative cleavage of the olefin moiety in **7** was carried out by employing the Lemieux–Johnson procedure,¹¹ which led to the production of the cyclic hemiacetal **5** in 62% yield through the intermediary dialdehyde **8**. The stereochemical issue with respect to the C8 position in **5** was assigned as depicted on the basis of NOESY experiment in the 500 MHz ¹H NMR spectrum.¹²

With the key intermediate **5** in hand, we next investigated the synthesis of the advanced key intermediate **3** having all four correct stereogenic centers (C1, C3, C3a, and C7a) and the required functionalities for elaboration to the target molecules **1** and **2** (Scheme 3). Thus, aryllithium **4** generated in situ by treatment of 4-bromo-1-methoxy-2-(methoxymethoxy)benzene (**9**)¹³ with *n*-butyllithium in THF at –78 °C was allowed to react with **5** at the same temperature, providing the coupling products **10a** (80%) and **10b** (20%) as

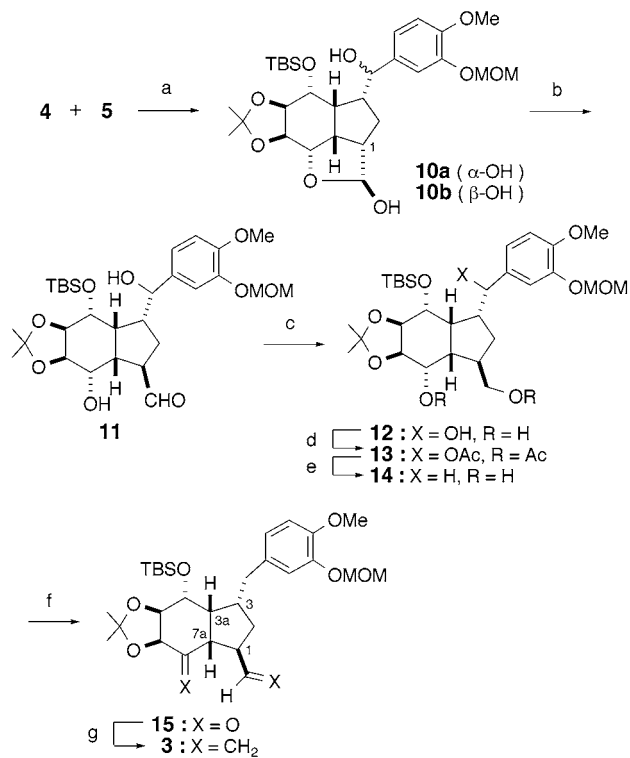
(10) (a) Izuwara, T.; Katoh, T. *Tetrahedron Lett.* **2000**, *41*, 7651. (b) Izuwara, T.; Katoh, T. *Org. Lett.* **2001**, *3*, 1653.

(11) Pappo, R.; Allen, D. S.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478.

(12) NOE between C8–H and C2–H_α was clearly observed.

(13) Compound **9** was prepared from commercially available 2-methoxyphenol via a four-step sequence of reactions [(a) BzCl, pyridine, rt, 98%; (b) Br₂, AcOH, rt; (c) K₂CO₃, MeOH, rt, 62% (two steps); (d) MOMCl, *i*-Pr₂NEt, CH₂Cl₂, rt, 88%] (see Supporting Information for experimental details).

Scheme 3. Synthesis of Key Intermediate **3**^a



^a Reagents and conditions: (a) 4-bromo-1-methoxy-2-(methoxymethoxy)benzene (**9**), *n*-BuLi, THF, $-78\text{ }^\circ\text{C}$; at $-78\text{ }^\circ\text{C}$ add **5**, 80% for **10a**, 20% for **10b**. (b) DBU, toluene, reflux, 30% (60% recovery of **10a**). (c) LiAlH_4 , THF, $0\text{ }^\circ\text{C}$, 96%. (d) Ac_2O , DMAP, pyridine, rt, 95%. (e) Li, liquid NH_3 , THF, $-78\text{ }^\circ\text{C}$, 98%. (f) Dess–Martin periodinane, CH_2Cl_2 , rt, 90%. (g) $\text{Ph}_3\text{P}^+\text{CH}_3\text{Br}^-$, *t*-BuOK, benzene, rt \rightarrow reflux, 95%.

a mixture of epimeric alcohols that were separated by column chromatography on silica gel.¹⁴ The crucial base-induced cyclic hemiacetal opening/epimerization of the liberated C1 formyl group in the major product **10a** turned out to be effected by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in refluxing toluene for 1.5 h, leading to the formation of the requisite product **11** (30%) along with the starting material **10a** (60%). Since prolonged reaction time caused decomposition of **11** and/or **10a**, the reaction was terminated when an approximately 1:2 mixture of **11**:**10a** was generated. After recycling of unreacted starting material **10a** four times, we could obtain $\sim 65\%$ yield of the desired compound **11**. Since the same treatment of the minor product **10b** turned out to be fruitless and resulted in recovery of the starting material, the projected synthesis was carried forward using only the major product **10a**.¹⁵ Compound **11** was further converted to the diol **14** in 89% overall yield via a three-step sequence involving reduction of the formyl group, complete acetylation of the three hydroxy groups in

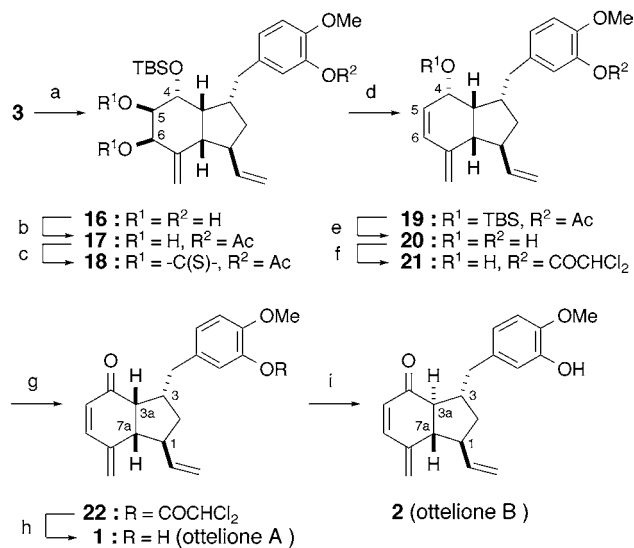
(14) Stereochemistry at the benzylic carbon in the coupling products **10a,b** was tentatively assigned on the basis of NOESY experiments in the ^1H NMR spectra.

(15) Detailed discussions on this hemiacetal opening/epimerization reaction will be presented in a full account.

12, and treatment of the resulting triacetate **13** with a large excess of lithium (100 equiv) in liquid ammonia at $-78\text{ }^\circ\text{C}$. Simultaneous oxidation of the primary and secondary hydroxy groups in **14** by the use of Dess–Martin periodinane furnished the corresponding keto-aldehyde **15** (90%), which was then subjected to twofold Wittig methylenation to provide the desired key intermediate **3** in 95% yield.

The final route that led to completion of the synthesis of the targeted otteliones A (**1**) and B (**2**) is shown in Scheme 4, which involves the critical elaboration of the highly

Scheme 4. Synthesis of Otteliones A (**1**) and B (**2**)^a



^a Reagents and conditions: (a) TFA, THF– H_2O , $0\text{ }^\circ\text{C}$, 86%. (b) Ac_2O , 2 M NaOH, *i*-PrOH, rt, 91%. (c) CSCl_2 , DMAP, CH_2Cl_2 , rt, 93%. (d) $(\text{EtO})_3\text{P}$, reflux, 78%. (e) TBAF, THF, rt, 83%. (f) $(\text{CHCl}_2\text{CO})_2\text{O}$, pyridine, CH_2Cl_2 , rt, 52%. (g) Dess–Martin periodinane, CH_2Cl_2 , rt, 95%. (h) 50% aq NaHCO_3 –MeCN (1:1), rt, 90%. (i) *t*-BuOK, *t*-BuOH, rt, 79% (**1**:**2** = 23:77 by ^1H NMR); isolation of **2** by HPLC, 23%.

sensitive 4-methylene-2-cyclohexenone system. Toward this end, deprotection of both the acetonide moiety and the MOM group in **3** by exposure to trifluoroacetic acid (TFA) in the presence of water followed by chemoselective acetylation of the phenolic hydroxy group in the resulting triol **16** afforded the acetate **17** in 78% yield for the two steps. To install the requisite C5–C6 double bond, we decided to employ a reliable Corey–Winter procedure.¹⁶ Thus, treatment of the diol **17** with thiophosgene in the presence of 4-(dimethylamino)pyridine (DMAP) provided the cyclic thiocarbonate **18** (93%), which was subsequently subjected to reductive elimination of the thiocarbonate moiety by reaction with triethyl phosphite, leading to the formation of the desired product **19** in 78% yield. Removal of the TBS and Ac protecting groups from **19** by treatment with tetrabutylammonium fluoride (TBAF) followed by chemose-

(16) (a) Corey, E. J.; Winter, R. A. E. *J. Am. Chem. Soc.* **1963**, *85*, 2677. (b) Corey, E. J.; Carey, F. A.; Winter, R. A. E. *J. Am. Chem. Soc.* **1965**, *87*, 934.

lective protection of the phenolic hydroxy group in the resulting diol **20** furnished the dichloroacetate **21**¹⁷ in 43% yield for the two steps. Finally, compound **21** was converted to ottelione A (**1**) in 86% overall yield via a two-step sequence involving Dess–Martin oxidation of the C4 hydroxy group in **21** and removal of the dichloroacetyl group in the resulting dienone **22**.

The spectroscopic properties (IR, ¹H and ¹³C NMR, MS) of the synthetic sample **1** were compatible with those of the natural product **1**.¹ Comparison of the optical rotation [synthetic **1**, [α]_D²⁵ +17.3° (*c* 0.55, CHCl₃); natural **1**, [α]_D²⁵ +14.0° (*c* 0.87, CHCl₃)¹⁸] established the absolute configuration of ottelione A (**1**) to be (1*S*,3*S*,3*aR*,7*aS*) as pictured in Scheme 4. Epimerization at C3*a* in ottelione A (**1**) to deliver ottelione B (**2**) was best carried out by exposure to *t*-BuOK in *t*-BuOH at room temperature for 2 h,¹⁹ leading to a 23:77 mixture of **1** and **2**. Isolation of **2** from this mixture was performed by means of HPLC (DAICEL CHIRALPAK AD-H) to yield an entirely pure sample of **2**, whose spectroscopic properties (IR, ¹H and ¹³C NMR, MS) were identical with those of natural **2**. The optical rotation of a pure synthetic sample of **2** [[α]_D²⁵ −333.0° (*c* 0.18, CHCl₃)]

(17) Selection of this dichloroacetyl protecting group was critical because this protecting group could be smoothly and cleanly removed without epimerization at the C3*a* position at the later stage (see **22** → **1**, Scheme 4). In our preliminary studies, we had prepared an *O*-acetyl variant of **22** (R = Ac); unfortunately, all attempts to remove the *O*-acetyl group from this compound resulted in partial epimerization at the C3*a* position. For accurately measuring the optical rotation of ottelione A (**1**), contamination with the C3*a* epimerized product [ottelione B (**2**)] should be prevented.

(18) Private communication from Professor Thomas R. Hoye of University of Minnesota. The value of optical rotation for natural otteliones A (**1**) and B (**2**) was not recorded in the literature cited in ref 1.

(19) Although efficient conversion of ottelione A (**1**) to ottelione B (**2**) has been previously demonstrated by Mehta (DBU, benzene, 65 °C, 83%),⁸ our initial attempts to follow the Mehta conditions for this conversion unfortunately resulted in the formation of an equilibrium mixture of **1** and **2** in a ratio of ca. 1:1.

was essentially identical to that of natural **2** (contaminated with a small amount of **1**, **2**:**1** = 85:15) [[α]_D²⁵ −276° (*c* 2.0, CHCl₃)¹⁸], indicating that natural **2** possesses the (1*S*,3*S*,3*aS*,7*aS*) absolute configuration as depicted in the formulation **2**.

In summary, the first enantioselective total synthesis of otteliones A (**1**) and B (**2**) has been achieved starting from the readily available tricyclic compound **6**. The absolute configuration of **1** and **2** was determined through the present synthesis. Our approach involves a coupling reaction of the cyclic hemiacetal **5** with aryllithium **4** (**4** + **5** → **10a,b**), base-induced cyclic hemiacetal opening/epimerization at the C1 position of **10a** (**10a** → **11**), and Corey–Winter's reductive elimination of the cyclic thiocarbonate **18** (**18** → **19**) as pivotal steps. The explored synthetic pathway should hold promise for preparing various ottelione analogues in enantiomerically pure form due to its generality and flexibility. These efforts are currently underway.

Acknowledgment. We are especially grateful to Professor Thomas R. Hoye (Department of Chemistry and Medicinal Chemistry, University of Minnesota) for providing us with copies of the IR, ¹H and ¹³C NMR, and MS spectra of natural otteliones A (**1**) and B (**2**) and for kindly informing us about the optical rotation of **1** and **2**. We also thank Dr. Naoki Sugimoto (National Institutes of Health Sciences) for measurements of HRMS.

Supporting Information Available: Detailed experimental procedures and full characterization data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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