A Route to Optically Pure (−)-Huperzine A: Molecular Modeling and in Vitro Pharmacology

Fumio Yamada,† Alan P. Kozikowski,*,† E. R. Reddy,‡ Yuan-Ping Pang,† Jacqueline H. Miller,‡ and Michael McKinney‡

Neurochemistry Research and Neuropharmacology Research, Mayo Clinic Jacksonville, 4500 San Pablo Road Jacksonville, Florida 32224

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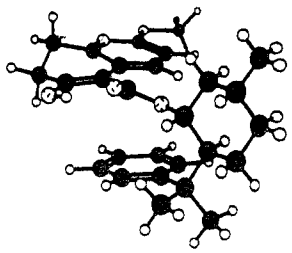
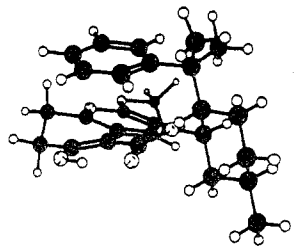
We have disclosed recently a total synthesis approach to (±)-huperzine A and its analogues.[1] Since both in vitro and in vivo pharmacological assays show that the racemate is half as potent as natural (−)-huperzine A,[1d] a result which would suggest that the (+)-isomer is poorly active as an inhibitor of acetylcholinesterase (AChE), we believed it important to develop an enantioselective approach to this valuable molecule.

On the basis of the route established to (±)-huperzine A, we chose to introduce absolute stereochemistry at the stage of the Michael/aldol (M-A) reaction which is used to create the bridging ring of huperzine A. Through the simple expedient of affixing a chiral auxiliary at the ester group of **1a**, we believed that an enantioselective approach to (−)-huperzine A could be found.[2] Prior to undertaking the laboratory work, molecular modeling studies were carried out in which the possible stereodirecting effects of different chiral auxiliaries were examined. The 8-phenylmenthol ester derivative **1b** emerged as an attractive candidate. In the molecular modeling study of **1b**, the global minimum energy conformation (gmec) and the low-lying minimum energy conformations (llmec) with either bottom-face shielding (bfs) or top-face shielding (tfs) were obtained by using the global searching method in MacroModel.[3] Assigning the gmec a relative population of 1.0, the relative populations of other llmecs were calculated according to the Boltzmann distribution law. A prediction of the ratio of the (−)- to (+)-isomers was then obtained from the ratio of the summed bfs populations to the summed tfs populations. At room temperature, a 4:1 ratio of (−)-isomer to (+)-isomer is predicted ($R_{predicted} = \Sigma n_- / \Sigma n_+ = 4/1$), assuming that the transition state for the chirogenic step mimics the geometries of the gmec or llmecs and that a reasonable level of "chirality preservation" occurs.[4] At lower temperatures (−20 °C), the ratio of (−)-huperzine A to (+)-huperzine A is expected to increase based upon the fact that the gmec yields (−)-huperzine A. A quantitative prediction of relative populations at lower temperatures cannot be made by the Boltzmann distribution law since it remains valid only if a fast exchange rate among the conformers exists.



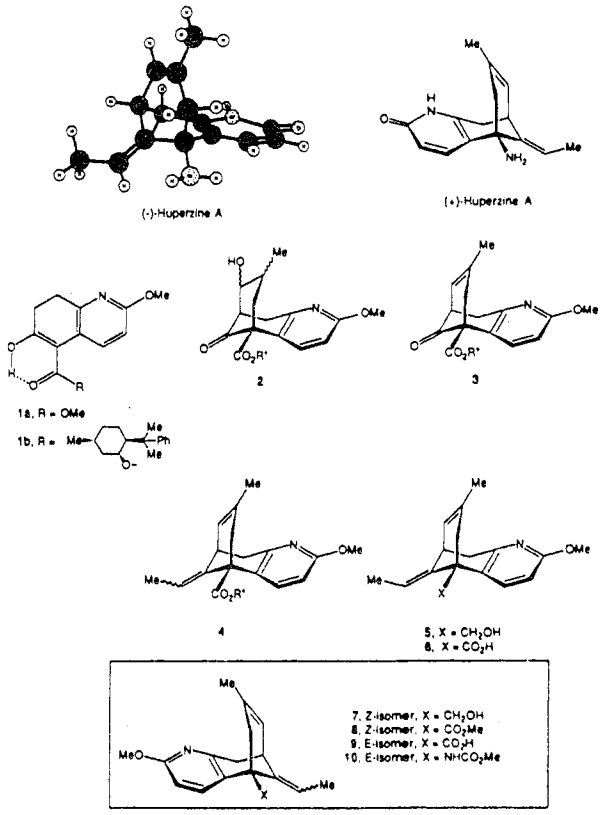
1b-1-(-), gmec leading to (-)-huperzine A



1b-1-(+), llmec leading to (+)-huperzine A

Figure 1.

Chart I



With this favorable prediction from the molecular modeling studies, **1a** was transesterified with (−)-8-phenylmenthol (benzene, reflux, 3 days, 91%), and **1b** reacted with methacrolein in the presence of tetramethylguanidine at room temperature over 2 days. A 90% yield of the mixture **2** was isolated. Mixture **2** was transformed to olefin **3** employing conditions identical with those reported previously.[1b] While yields for the elimination step are modest, the ratio of chromatographically separable olefinic diastereomers was readily assigned by ^{1}H NMR analysis and found to be 4:1, *a ratio identical with that predicted above*. If the M-A

† Neurochemistry Research.
‡ Neuropharmacology Research.
‖ Present address: SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406.

(1) (a) Xia, Y.; Reddy, E. R.; Kozikowski, A. P. *Tetrahedron Lett.* **1989**, *30*, 3291. (b) Xia, Y.; Kozikowski, A. P. *J. Am. Chem. Soc.* **1989**, *111*, 4116. (c) Hanin, I.; Tang, X. C.; Corey, J.; Xia, Y.; Reddy, E. R.; Kozikowski, A. P. *The FASEB J.* **1990**, *4*, A471. (d) Kozikowski, A. P.; Thiels, E.; Tang, X. C.; Hanin, I. In *Advances in Medicinal Chemistry*; Maryanoff, B. E., Maryanoff, C. A., Eds.; JAI Press, in press.

(2) For other examples of asymmetric induction in the Michael reaction, see, inter alia: Pfau, M.; Revial, G.; Guingant, A.; d'Angelo, J. *J. Am. Chem. Soc.* **1985**, *107*, 273. Sevin, A.; Tortajada, J.; Pfau, M. *J. Org. Chem.* **1986**, *51*, 2671. Volpe, T.; Revial, G.; Pfau, M.; d'Angelo, J. *Tetrahedron Lett.* **1987**, *28*, 2367. Hirai, Y.; Terada, T.; Yamazaki, T. *J. Am. Chem. Soc.* **1988**, *110*, 958. d'Angelo, J.; Guingant, A.; Riche, C.; Chiaroni, A. *Tetrahedron Lett.* **1988**, *29*, 2667. d'Angelo, J.; Revial, G.; Volpe, T.; Pfau, M. *Tetrahedron Lett.* **1988**, *29*, 4427. Desmaele, D.; d'Angelo, J. *Tetrahedron Lett.* **1989**, *30*, 345. d'Angelo, J.; Revial, G.; Guingant, A.; Riche, C.; Chiaroni, A. *Tetrahedron Lett.* **1989**, *30*, 2645. Aoki, S.; Sasaki, S.; Koga, K. *Tetrahedron Lett.* **1989**, *30*, 7229.

(3) We thank Professor W. C. Still, Department of Chemistry, Columbia University, New York, for a copy of *MacroModel Version 2.5*. Energy minimizations were performed with use of the BDNR method and the MM2 force field. Global searching was carried out in the MULTIC submode.

(4) The observation that the menthol ester derivative of **1a** leads to a 1:1 mixture of olefinic diastereomers underscores the importance of off-center π-stacking to the asymmetric induction step as well as provides evidence that the ratios reported here reflect the chirogenic nature of the Michael addition step and *not* the stereochemical course of the dehydration step.

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reaction is run at −20 °C, the ratio of olefin isomers secured upon dehydration is improved to 9:1.[4] For the major olefin isomer **3**, further steps in its transformation to huperzine A were similar to those reported previously. In the present work, however, the PhSH/AIBN catalyzed isomerization of the 10:1 *Z*/*E* mixture of olefin isomers **4** proved less efficient.[5] After two repetitions of the isomerization reaction, the 10:1 mixture was converted to but a 1:1.4 *Z*/*E* mixture. After chromatographic separation, the *E* isomer was reacted with LAH (THF, room temperature, 7 h, 84%) to give alcohol **5** which was oxidized to acid **6** by Jones reagent (room temperature, 1 h, 90%). Subsequent Curtius rearrangement of the acid **6** ($SOCl_2$, $PhCH_3$, 75–80 °C, 1.5 h; NaN_3, $PhCH_3$, 75–80 °C, 8 h; MeOH, reflux, 14 h, 80% overall) then led to huperzine A which exhibited an $[\alpha]^{25}_D$ of −147°, a value nearly identical with that measured for natural (−)-huperzine A [$[\alpha]^{25}_D$ −150° (*c* 0.12, $CHCl_3$)].[6]

On taking the minor diastereomer **3′** through the same sequence of reactions, small amounts of (+)-huperzine A could be obtained. To obtain larger quantities of (+)-huperzine A, it was more efficient to react (±)-**7** with (*S*)-MTPA-Cl (Et_3N, CH_2Cl_2, room temperature, 2 h) and to separate the resulting diastereomers by column chromatography. The resulting optically pure ester was reduced with LAH/Et_2O to provide optically active **7**. Jones oxidation (acetone, 27 °C, 1.5 h) followed by esterification with methyl iodide (DBU, CH_3CN, room temperature, 2 h, 46% overall) provided ester **8**. Next, **8** was isomerized to the (*E*)-olefin with thiophenol/AIBN in toluene, and the ester hydrolyzed to acid **9** with 20% aqueous NaOH in MeOH–THF at reflux (73% overall). Acid **9** was transformed to the carbamate **10** [$(PhO)_2P(O)N_3$, Et_3N, $PhCH_3$, reflux, 2.5 h; MeOH, reflux, 18 h, 63% overall) which was deprotected in the standard way (TMSI; MeOH, 85%) to furnish (+)-huperzine A of $[\alpha]^{25}_D$ +147° (*c* 0.72, $CHCl_3$).[7]

To examine the in vitro biological activity of (+)-huperzine A, the compound was tested over a concentration range of 10–1000 nM for its ability to inhibit AChE from rat cortex.[8] The IC_{50} for (+)-huperzine A was found to be 1448 ± 62.4 nM (n = 5), which is 33-fold larger than that of (−)-huperzine A (IC_{50} = 44.5 ± 2.9 nM (n = 3)). Racemic huperzine A has an IC_{50} of 71.5 ± 2.4 nM (n = 7).

The difference in IC_{50}'s of the pure enantiomers demonstrates a reasonably large stereoselectivity of action for huperzine A. Nonetheless, this difference is not as great as that reported for physostigmine wherein the (+)-isomer is over 700 times less potent than its (−)-isomer in inhibiting AChE from the cortex.[9] Such differences probably reflect the more critical positioning required of the physostigmine molecule as a consequence of its inhibitory action being due to its ability to carbamoylate the enzyme, a feature not exhibited by the huperzine molecule.[10]

The present work has important implications for the use of huperzine A in the palliative treatment of Alzheimer's disease. Further applications of the chemistry described herein to the preparation of optically pure analogues of huperzine A for evaluation as cognition enhancers are being explored.

Acknowledgment. We are indebted to the National Institute on Aging (Grant No. 1RO1AG07591) for their generous support of our program.

(5) Bhalerao, U. T.; Rapoport, H. *J. Am. Chem. Soc.* **1971**, *93*, 4835.

(6) Due to our observation that both natural and synthetic (−)-huperzine A formed a precipitate in MeOH, the solvent employed in the determination of the published optical rotation for the natural compound, our rotations were measured in $CHCl_3$. See: Liu, J. S.; Zhu, Y. L.; Yu, C. M.; Zhou, Y. Z.; Ha, Y. Y.; Wu, F. W.; Qi, B. F. *Can. J. Chem.* **1986**, *64*, 837.

(7) Chiral hplc analysis reveals that (+)-huperzine A is 99.1% pure and contaminated by (−)-huperzine A. The IC_{50} for (+)-huperzine A will therefore be slightly larger than the value presented above.

(8) Wilson, S. H.; Schrier, B. K.; Farber, J. L.; Thompson, E. J.; Rosenberg, R. W.; Blume, A. J.; Nirenberg, M. W. *J. Biol. Chem.* **1972**, *147*, 3159.

(9) Atack, J. R.; Yu, Q. S.; Soncrant, T. T.; Brossi, A.; Rapoport, S. I. *J. Pharmacol. Exp. Ther.* **1989**, *249*, 194. Brossi, A. *J. Med. Chem.* **1990**, *33*, 2311.

(10) Spero, L. In *Principles of Medical Pharmacology*; Kalant, H., Rochlau, W. H. E., Eds.; B. C. Decker Inc.: Toronto, 1989; pp 141–147.

Supplementary Material Available: Table I containing relative populations and MM2 energies of tfs and bfs conformations and full experimental details including spectral data for the synthesis of (−)-huperzine A (13 pages). Ordering information is given on any current masthead page.

Use of Hydrogen Bonds to Control Molecular Aggregation. Self-Assembly of Three-Dimensional Networks with Large Chambers

Michel Simard, Dan Su, and James D. Wuest*

Département de Chimie, Université de Montréal Montréal, Québec, H3C 3J7 Canada

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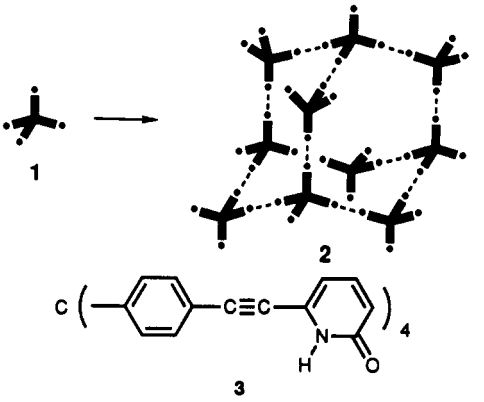
Noncovalent interactions that are selective, directional, and strongly attractive can induce the self-assembly of predictable supramolecular aggregates. We have shown that the tendency of 2-pyridones to form hydrogen-bonded dimers allows them to be used as sticky sites that compel molecules to associate, thereby driving the self-assembly of aggregates joined by extensive networks of hydrogen bonds.[1,2] This work suggested that the creative incorporation of multiple sticky sites in rigid frameworks might induce the self-assembly of three-dimensional networks with internal chambers. For example, hypothetical compound **1** should be forced by its tetrahedral geometry and the presence of four sticky sites (·) to form the cubic diamondoid network **2** or a related hexagonal lonsdaleite lattice. In this communication, we show



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that the self-assembly of such structures is possible; under suitable conditions, self-association of rigid tetrapyridone **3** produces an organic diamondoid network **2** with large internal chambers that selectively enclathrate molecules present during the self-assembly.[3,4]

(1) (a) Gallant, M.; Phan Viet, M. T.; Wuest, J. D. *J. Org. Chem.* **1991**, *56*, 2284–2286. (b) Ducharme, Y.; Wuest, J. D. *J. Org. Chem.* **1988**, *53*, 5787–5789.

(2) For recent related work, see: Tecilla, P.; Dixon, R. P.; Slobodkin, G.; Alavi, D. S.; Waldeck, D. H.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 9408–9410. Zerkowski, J. A.; Seto, C. T.; Wierda, D. A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 9025–9026. Etter, M. C.; Urbañczyk-Lipkowska, Z.; Zia-Ebrahimi, M.; Panunto, T. W. *J. Am. Chem. Soc.* **1990**, *112*, 8415–8426. Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024–8034. Zhao, X.; Chang, Y.-L.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6627–6634. Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249–1250. Lehn, J.-M.; Mascal, M.; DeCian, A.; Fischer, J. *J. Chem. Soc., Chem. Commun.* **1990**, 479–481.

(3) The self-assembly of networks with large cavities can also be induced by coordination to metals. Gable, R. W.; Hoskins, B. F.; Robson, R. *J. Chem. Soc., Chem. Commun.* **1990**, 762–763. Hoskins, B. F.; Robson, R. *J. Am. Chem. Soc.* **1990**, *112*, 1546–1554. For other recent work, see: Nenoff, T. M.; Harrison, W. T. A.; Gier, T. E.; Stucky, G. D. *J. Am. Chem. Soc.* **1991**, *113*, 378–379. Mundi, L. A.; Strohmaier, K. G.; Goshorn, D. P.; Haushalter, R. C. *J. Am. Chem. Soc.* **1990**, *112*, 8182–8183. Fujita, M.; Yazaki, J.; Ogura, K. *J. Am. Chem. Soc.* **1990**, *112*, 5645–5647. Adam, M.; Brimah, A. K.; Fischer, R. D.; Li, X.-F. *Inorg. Chem.* **1990**, *29*, 1595–1597. Saalfrank, R. W.; Stark, A.; Bremer, M.; Hummel, H.-U. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 311–314.

0002-7863/91/1513-4696$02.50/0