A Concise Synthesis of Ginkgolide M, a Minor Component of a Terpene Trilactone Fraction from *Ginkgo biloba* Roots[⊥]

Sergei Bolshakov, Sergei V. Dzyuba,* John Decatur, and Koji Nakanishi*

Department of Chemistry, Columbia University, 3000 Broadway, New York, New York 10027

Received October 14, 2005

Ginkgolide M (**GM**), which is found only in the roots of the *Ginkgo biloba* tree and is an inhibitor of ligand-operated ion channels in the central nervous system, has been prepared in three steps from 10-benzylginkgolide C, an intermediate generated during the isolation and separation of ginkgolides from *Ginkgo biloba* leaf extract. The described synthetic sequence can be applied to access **GM** derivatives for biological studies.

Ginkgo biloba leaf extract, an over-the-counter dietary supplement or prescription product with multimillion dollar worldwide sales, has long been advocated for its ability to improve memory in the elderly.[1] The potential of *Ginkgo biloba* leaf extract for the treatment of cognitive impairment has been demonstrated in an array of studies.[1,2] However, neurophysiological effects and long-term neuroprotective properties of the extract still need to be clarified.

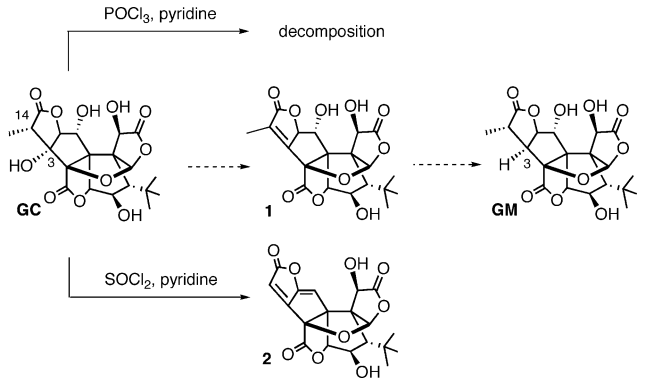
Activation of ligand-gated ion channels and G-protein coupled receptors is responsible for many processes, such as basal excitatory synaptic transmission and many forms of synaptic plasticity that are thought to control memory and learning.[3] Ligand-gated ion channels, including glycine, $GABA_A$, NMDA, and AMPA receptors, are involved in mediation of excitatory synaptic transmission, a process by which neurons communicate with each other.[3] Small molecules that can modulate activity of these receptors are valuable as mechanistic probes for the processes in the central nervous system as well as pharmacological leads for developing remedies for neurological disorders.

Although the molecular mechanisms of *G. biloba* extract components are largely unknown, they are often attributed to the presence of structurally unique compounds named ginkgolides (Figure 1).[4] Ginkgolides are believed to be responsible for a variety of neuromodulatory effects exhibited by *G. biloba* leaf extract, including learning and memory functions.[5] Several studies addressed structure−activity relationships of ginkgolides toward platelet-activating factor[6] and glycine[7] receptors and have indicated a very fine balance between the number and position of the hydroxy groups around the ginkgolide skeleton and biological activity.

It was recently demonstrated that ginkgolides could block and modulate the responses of several ion channel receptors.[7a] Noteworthy, **GM**, which is found only in the root of *Ginkgo biloba* L. (Ginkgoaceae), unlike other ginkgolides that are found in the leaves as well, was shown to be the most potent natural ginkgolide in blocking the responses of several receptor-gated channels, whereas other tested ginkgolides (**GA**, **GB**, **GC**, and **GJ**) showed antagonistic properties exclusively toward glycine receptor. In particular, of all the ginkgolides, **GM** exhibited the highest inhibition of $GABA_A$ receptor and efficiently displaced TBPS[8] from the convulsant binding site of $GABA_A$.[7a] Ion channel blocker properties make **GM** a lead for potential treatment of neurodegenerative disorders, such as Alzheimer's disease.[9]

From a structural point of view, **GM** lacks the tertiary hydroxy group at the C-3 position, which is present in other ginkgolides from *G. biloba* extract, and, therefore, represents a unique analogue

Scheme 1. Attempted 3,14-Dehydration of GC



to address the effect of subtle structural changes on ginkgolide−receptor interactions. Yet the biological scope and potential of this ginkgolide is not broadly studied; apparently, the available quantities of **GM** are relatively small as compared to other ginkgolides, thus making structure−activity relationship studies quite challenging. Therefore, a practical synthetic preparation of **GM** is desirable.[10] Dehydration of OH-3 from unprotected **GC** would create a plausible intermediate, such as **1**, en route to efficient synthesis of **GM** (Scheme 1). However, subjection of **GC** to known dehydration procedures[10] led to either decomposition of the starting material or formation of the double-dehydrated product **2** (Scheme 1).[11]

During the course of studies directed toward regiocontrolled synthesis of fluorinated ginkgolides, we found that treatment of **GA** with (diethylamino)sulfur trifluoride (DAST)[12] provided no fluorodehydroxylation at the C-10 position, but instead led to a high yield selective elimination of the tertiary hydroxy group, OH-3, affording **GL** (Scheme 2).[13] Upon hydrogenation of the unsaturated trilactone moiety of **GL** in the presence of Crabtree's catalyst,[14] a clean formation of *epi*-derivative **3** took place (Scheme 2). The *cis* orientation of H-3 and H-14 in 3-dehydroxy-14-*epi*-**GA**, **3**, was confirmed by NOE studies: an NOE was observed between the 14-Me and 12-H, which indicated that the 14-Me and 3-H are in a *trans* orientation; the 14-Me extends back toward the backbone, i.e., "β-oriented" (Scheme 2).[15]

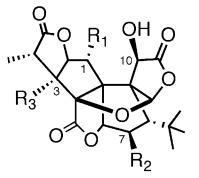
Attempts to achieve dehydration of **GC** upon reaction with DAST under the conditions outlined in Scheme 2 led to decomposition of the starting material.[16] No desired elimination product was obtained when the reaction was conducted at different temperatures (−78 and 0 °C); instead, the starting material was recovered. Application of bases, i.e., pyridine and 4-(dimethylamino)pyridine (DMAP), to facilitate the dehydration process[17] was also unsuccessful.

Since extra hydroxy groups of **GC** (as compared to **GA** and **GB**) are likely to contribute to the inefficiency of the dehydration, we turned our attention to monoprotected **GC** analogues. It is relevant

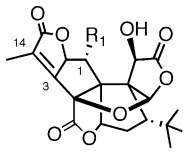
[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed. (S.V.D.) Tel: +1 212 854 5356. Fax: +1 212 932 1289. E-mail: svd2102@columbia.edu. (K.N.) Tel: +1 212 854 2169. Fax: +1 212 932 1289. E-mail: kn5@columbia.edu.

10.1021/np050403i CCC: $33.50
Published on Web 02/22/2006



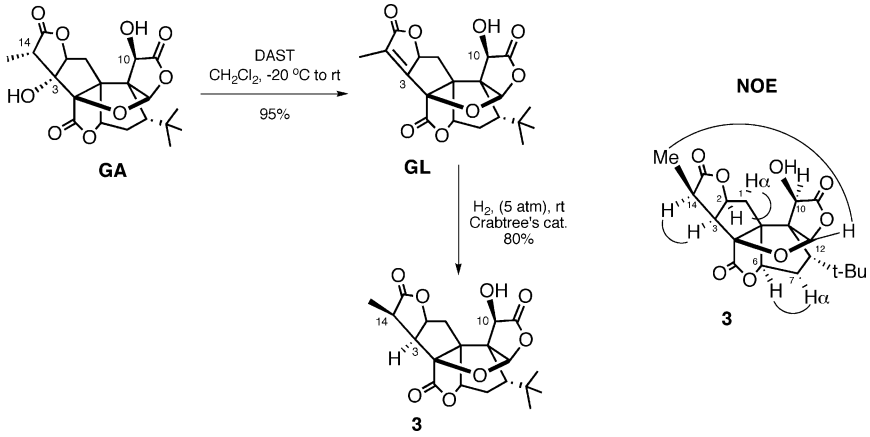
	R_1	R_2	R_3
Ginkgolide A (**GA**)	H	H	OH
Ginkgolide B (**GB**)	OH	H	OH
Ginkgolide C (**GC**)	OH	OH	OH
Ginkgolide J (**GJ**)	H	OH	OH
Ginkgolide M (**GM**)	OH	OH	H



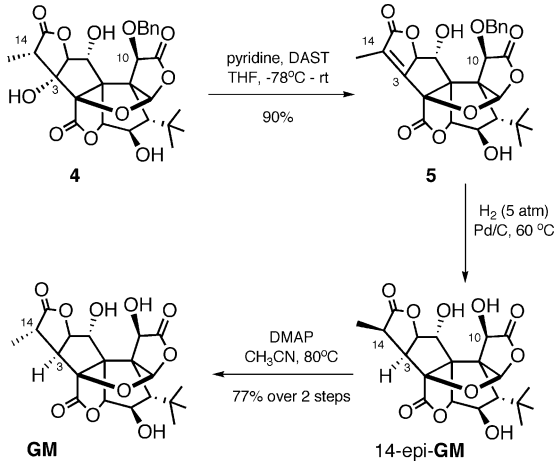
	R_1
Ginkgolide L (**GL**)	H
Ginkgolide K (**GK**)	OH

Figure 1. Ginkgolide structures from *Ginkgo biloba* (leaves and roots).

Scheme 2. DAST-Mediated GA to GL Conversion and Stereochemical Assignment of Hydrogenated GL



Scheme 3. Synthesis of GM



to note that the 10-benzyl derivatives of **GB** and **GC** are intermediates prepared for the separation of individual ginkgolides from *G. biloba* leaf extract.[18] Therefore, 10-benzyl-**GC** is an attractive starting point to explore the synthesis of ginkgolides and their derivatives. It was found that the reaction of 10-benzyl-**GC 4** with DAST in the presence of pyridine in THF led to a clean elimination of the OH-3 group, giving unsaturated lactone **5** in good yield (Scheme 3).[19] Hydrogenation of the unsaturated lactone moiety yielded the known 14-*epi*-**GM**, which in turn was converted into **GM** under previously reported conditions.[10]

In conclusion, a short synthesis of **GM** has been achieved, which features selective removal of the tertiary hydroxy group in the presence of two unprotected secondary alcohol moieties and does not require the use of isolated **GC**. Thus the whole process can be achieved in a few steps starting from the commercial *G. biloba* leaf extract.[20]

Experimental Section

General Experimental Procedures. All reagents and solvents were purchased from Aldrich and used as received. Ginkgolides were available from earlier studies or isolated from BioGinkgo 27/7 extract according to a literature procedure.[17,20] ^{1}H NMR spectra were recorded on a Bruker DPX-300 (300 MHz) spectrometer and are reported in ppm from $CDCl_3$ internal standard (7.26 ppm). 2D NOESY spectra were acquired on a Bruker DMX 500 spectrometer under the following conditions: TPPI mode; SW = 3500 Hz; TD2 = TD1*4 = 1200; D1 = 5 s; NS = 40. For the NOESY volume ratio analysis, several different reference distances and NOEs were used, and the choice did not affect the calculated distances significantly.

Example of Dehydration Procedure. 10-Benzyl-**GC 4** (15.0 mg, 0.029 mmol) was dissolved in 1 mL of THF and cooled to −78 °C. Pyridine (100 μL, 1.23 mmol) and DAST (100 μL, 0.76 mmol) were added dropwise at this same temperature. The reaction mixture was stirred at −78 °C for 10 min, warmed to room temperature, and then kept for 40 min before quenching by addition of 2 mL of water. The aqueous layer was extracted with 3 × 2 mL of EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo. Purification by flash chromatography on silica gel (eluted with 1:1 EtOAc/hexane) afforded the desired 3,14-dehydro-10-benzyl-**GC 5** (13.1 mg, 0.026 mmol, 90% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (5H, m), 6.00 (s, 1H), 5.85 (1H, s), 5.42 (1H, d, J = 10.2 Hz), 4.82 (1H, s), 4.63 (1H, d, J = 10.3 Hz), 4.60 (1H, d, J = 5.0 Hz), 4.39 (1H, m), 2.20 (3H, s), 2.03 (2H, m), 1.92 (1H, d, J = 12.4 Hz), 1.23 (9H, s).

Synthesis of GM. A glass liner for a stainless steel 45 mL Parr high-pressure reactor equipped with a stir bar was charged with a solution of 3,14-dehydro-10-benzyl-**GC 5** (12.0 mg, 0.024 mmol) in a mixture of EtOAc (1 mL) and MeOH (3 mL), then Pd/C (10% w/w, 2 mg) was added. The liner was inserted into the Parr reactor, and the

pressure gauge and gas assembly were attached. The reactor was sealed, charged and vented with 3 × 400 psi with H_2, and recharged to 600 psi H_2. The reaction mixture was stirred at room temperature for 18 h, and then the reactor was vented. The solution was filtered through a pad of Celite and concentrated in vacuo. The residue was dissolved in 1 mL of MeCN, and DMAP (12.0 mg, 0.096 mmol) and 0.1 mL of water were added. The reaction mixture was stirred 80 °C in sealed tube for 4 days and then quenched by addition of 2 mL of 1 M HCl. The aqueous layer was extracted with 3 × 2 mL of EtOAc, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo. Purification by flash chromatography on silica gel (eluted with 2:1 EtOAc/hexane) afforded **GM** (7.9 mg, 0.019 mmol, 77% yield over two steps), whose spectral data matched those of the natural compound.[4a]

Acknowledgment. We are grateful to Pharmanex for a generous gift of *Ginkgo biloba* leaf extract. This work was financially supported by NIH (GM-MH068817).

References and Notes

(1) Stromgaard, K.; Vogensen, S. B.; Nakanishi, K. In *Encyclopedia of Dietary Supplements*; Coates, P. M., Ed.; Marcel Dekker Inc.: New York, 2005; pp 249−257.

(2) (a) van Dongen M. C. J. M.; van Rossum, E.; Knipschild, P. In *Ginkgo biloba;* van Beek, T. A., Ed.; Harwood Academic Publishers: Australia, 2000; Chapter 22, pp 385−442. (b) Crews, W. D., Jr.; Harrison, D. W.; Griffin, M. L.; Falwell, K. D.; Crist, T.; Longest, L.; Hehemann, L.; Rey, S. T. *HerbalGram* **2005**, *67*, 43−62.

(3) (a) Guderman, T.; Schoneberg, T.; Schultz, G. *Annu. Rev. Neurosci.* **1997**, *20*, 399−427. (b) Ito, M, *Physiol. Rev.* **2001**, *81*, 1143−1195. (c) El Far, O.; Betz, H. *Biochem. J.* **2002**, *365*, 329−336. (d) Debanne, D.; Daoudal, G.; Sourdet, V.; Russier, M. *J. Physiol.* **2004**, *97*, 403−414. (e) Malbon, C. C. *Nature Rev. Mol. Cell Biol.* **2005**, *6*, 689−701. (f) Lujan, R.; Shigemoto, R.; Lopez-Bendito, G. *Neuroscience* **2005**, *130*, 567−580.

(4) (a) van Beek, T. A. *Bioorg. Med. Chem.* **2005**, *13*, 5001−5012. (b) **GK** and **GL** were identified as new ginkgolides present in trace amounts in the terpene trilactone fraction of *Ginkgo biloba* leaf extract: Wang, Y.; Sheng, L. S.; Lou, F. C. *Acta Pharmcol. Sin.* **2001**, *31*, 606−608.

(5) (a) Nakanishi, K. *Bioorg. Med. Chem.* **2005**, *13*, 4983−5000. (b) Stromgaard, K.; Nakanishi, K. *Angew. Chem., Int. Ed.* **2004**, *43*, 1640−1658.

(6) (a) Hosford, D.; Mencia-Huerta, J. M.; Page, C.; Braquet, P. *Phytotherapy Res.* **1988**, *2*, 1−24. (b) Hu, L.; Chen, Z.; Xie, Y.; Jiang, Y.; Zhen, H. *Bioorg. Med. Chem.* **2000**, *8*, 1515−1521. (c) Vogensen, S. B.; Stromgaard, K.; Shindou, H.; Jaracz, S.; Suehiro, M.; Ishii, S.; Shimizu, T.; Nakanishi, K. *J. Med. Chem.* **2003**, *46*, 601−608. (d) Zhu, W.; Chen, G.; Hu, L.; Luo, X.; Gui, C.; Luo, C.; Puah, C. M.; Chen, K.; Jiang, H. *Bioorg. Med. Chem.* **2005**, *13*, 313−322.

(7) (a) Chatterjee, S. S.; Kondratskaya, E. L.; Krishtal, O. A. *Pharmacopsychiatry* **2003**, *36* (Suppl. 1), S68−S77. (b) Ivic, L.; Sands, T. T. J.; Fishkin, N.; Nakanishi, K.; Kriegstein, A. R.; Stromgaard, K. *J. Biol. Chem.* **2003**, *278*, 49279−49285. (c) Jaracz, S.; Nakanishi, K.; Jensen, A. A.; Stromgaard, K. *Chem. Eur. J.* **2004**, *10*, 1507−1518. (d) Kondratskaya, E. L.; Betz, H.; Krishtal, O. A.; Laube, B. *Neuropharmacology* **2005**, *49*, 945−951.

(8) TBPS: ^{35}S-*tert*-butylbicyclophosphorothionate. Edgar, P. P.; Schwartz, R. D. *J. Neurosci.* **1990**, *10*, 603−612.

(9) Schmitt, H. P. *Med. Hypothesis* **2005**, *65*, 259−265.

(10) Synthetic studies leading to formation of **GM** were reported previously: (a) Weinges, K.; Rummler, M.; Schick, H. *Liebigs Ann. Chem.* **1993**, 1023−1027. (b) Maruyama, M.; Terahara, A.; Nakadaira, Y.; Woods, M. C.; Takagi, Y.; Nakanishi, K. *Tetrahedron Lett.* **1967**, *8*, 315−319.

(11) Maruyama, M.; Terahara, A.; Nakanishi, K. *Sci. Rep. Tohoku Univ.* **1967**, *50*, 92−99.

(12) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574−578.

(13) **GA** to **GL** conversion using $POCl_3$/pyridine was previously reported: Weinges, K.; Rümmler, M.; H. Schick, H.; Schilling, G. *Leibigs Ann. Chem.* **1993**, 287−291.

(14) Attempts to achieve the desired hydrogenation using Pd/C failed. Upon screening several hydrogenation catalysts, Crabtree's catalyst was identified as the most efficient. For a review of Crabtree's catalysts see: Grabtree, R. H. *Acc. Chem. Res.* **1990**, *23*, 95−101.

(15) Further proof of stereochemistry is as follows: the 2D NOESY cross-peak volumes were analyzed using a NOE ratio method upon which the volume of the Me-14/H-12 cross-peak yielded a distance of 0.35 nm, consistent with the *cis* orientation of H-3 and H-14. The *cis* orientation of H-2 and H-3 was supported by NOESY volume analysis. The cross-peak volume between H-2 and H-3 was similar in magnitude to the peak volume for several other *cis*-oriented protons, such as between the H-6 and H-7α protons and between the H-2 and H-1α protons. For the NOE ratio method see: Newhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, 2nd ed.; Wiley-VCH: New York, 2000.

(16) **GB**, however, was successfully converted into **GK**, using a DAST-mediated protocol, Scheme 2.

(17) Somekh, L.; Shanzer, A. *J. Org. Chem.* **1982**, *48*, 907−908.

(18) Jaracz, S.; Malik, S.; Nakanishi, K. *Phytochemistry* **2004**, *65*, 2897−2902.

(19) The DAST-mediated procedure appears to be quite general for the dehydration of ginkgolides and ginkgolide derivatives, as 10-benzyl-**GB** and 10-methyl-**GC** underwent a clean elimination of the OH-3 group, yielding the corresponding 3,14-unsaturated products in 90 and 85% yields, respectively.

(20) All ginkgolides can be selectively isolated from the *Ginkgo biloba* leaf extract in one step: Nakanishi, K.; Jaracz, S.; Malik, S.; Ishii, H.; Dzyuba, S. V. PCT Int. Appl. WO 2005046829, 2005, 61 pp.

NP050403I