Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5673–5675
Bioorganic & Medicinal Chemistry Letters

Preparation of a tritiated ginkgolide☆

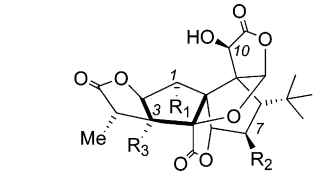
Kristian Strømgaard,*,† Makiko Suehiro and Koji Nakanishi*

Department of Chemistry, Columbia University, New York, NY 10027, USA

Received 18 June 2004; revised 17 August 2004; accepted 18 August 2004
Available online 15 September 2004

Abstract—Ginkgolide B, a constituent of the tree *Ginkgo biloba*, was radiolabeled with the β-emitter tritium ([^{3}H]) in two steps from ginkgolide C. First, a triflate precursor was prepared utilizing the selective reactivity of 7-OH in ginkgolide C; the triflate was then reduced with sodium borotritide to yield tritiated ginkgolide B ([^{3}H]GB) in good yield and high specific activity. The tritiated ginkgolide will be an important tool for studying neuromodulatory properties of ginkgolides.

Ginkgo biloba L. is the last surviving member of a family of trees, Ginkgoacea that appeared more than 250 million years ago and has been mentioned in the Chinese Materia Medica for more than 2500 years.[1] A number of *G. biloba* natural products have been isolated, the most unique being the terpene trilactones, that is, ginkgolides A, B, C, J, and M (**1**–**5**, Fig. 1) and bilobalide.[2] The ginkgolides are diterpenes with a cage skeleton consisting of six 5-membered rings, a spiro[4.4]nonane carbocyclic ring, three lactones, and a tetrahydrofuran. The difference of the five ginkgolides is variations in the number and positions of hydroxyl groups at C-1, C-3, and C-7 of the spirononane framework (Fig. 1). A standardized *G. biloba* extract, EGb 761 containing terpene trilactones (5–7%) and flavonoids (22–24%), has demonstrated neuromodulatory properties,[3] while several clinical studies using EGb 761 have confirmed positive effects on various neurodegenerative diseases.[4] Recent studies on healthy volunteers have also shown positive effects of EGb 761 on short-term working memory.[5]



	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

Figure 1. Structures of the five ginkgolides isolated from *Ginkgo biloba*.

Although ginkgolides have been shown to be neuroprotective in vitro, the molecular basis for the action of *G. biloba* constituents on the central nervous system (CNS), in particular terpene trilactones, is poorly understood.[6] Moreover, ginkgolide B (GB, **2**) is a potent in vitro antagonist of the platelet-activating factor receptor (PAFR), a G protein-coupled receptor[7] that is a potential target for neurodegenerative diseases.[8] PAF has been suggested as a retrograde messenger in long-term potentiation (LTP), although this role remains controversial.[9] Thus, PAFR plays an important role in the brain, but whether the effect of ginkgolides in the CNS is related to its effect on PAFR, or whether ginkgolides have other targets in the CNS is currently unknown.[10]

Recently, a new target for ginkgolides has been discovered; ginkgolides are highly potent and selective antagonists of glycine receptors, which belong to the ligand-gated ion channel (LGIC) family.[11] The

Keywords: Natural products; Radiolabeling; Ginkgolides.

☆ Part of this work was presented at the 15th International Symposium on Radiopharmaceutical Chemistry, August 10–14, 2003, Sydney, Australia. Abstract published in *J. Labelled Compd. Radiopharm.* **2003**, *46*, S214.

* Corresponding authors. Tel.: +1 212 854 2169; fax: +1 212 932 8273; e-mail: kn5@columbia.edu

† Present address: Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

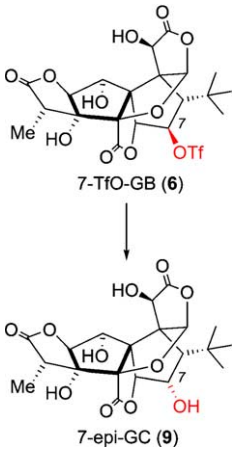
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.08.042

importance of this action in relation to neuromodulatory properties of ginkgolides has yet to be characterized, and a radiolabeled ginkgolide becomes a vital tool for such studies.

In order to explore targets for ginkgolides in CNS on a molecular level we have prepared a tritiated ginkgolide where the ginkgolide skeleton has been labeled with the long-lived β-emitter tritium ([^{3}H]). The [^{3}H]-labeled ligand can be used for ex vivo autoradiography, radioligand binding assays, and other in vitro studies with various cell cultures. Tritiated ginkgolides will provide information on whether ginkgolides cross the blood brain barrier (BBB), the distribution of specific binding sites in the CNS, and in particular whether or not ginkgolides have specific binding sites other than the PAFR.

Previous studies have reported approaches related to radiolabeling of *G. biloba* constituents, primarily with [^{14}C]; in one study leaves from *G. biloba* plants fed with [^{14}C]-acetate was extracted, leading to a [^{14}C]-labeled *G. biloba* extract, however, the actually labeled chemical constituents are not identified.[12] Recently, an approach to the synthesis of [^{14}C]-labeled ginkgolide A (GA, **1**) using [^{14}C]-labeled methyl propionate, was reported, but synthesis of the actual radiolabeled ligand was not described.[13] Moreover, the application of a [^{14}C]-labeled ginkgolide may have serious limitations because of its low specific activity. Very recently ginkgolide B has been labeled with the positron emitter ^{18}F to visualize its in vivo behavior by positron emission tomography (PET).[14] Here we describe the synthesis of high specific activity [^{3}H]-ginkgolide B.

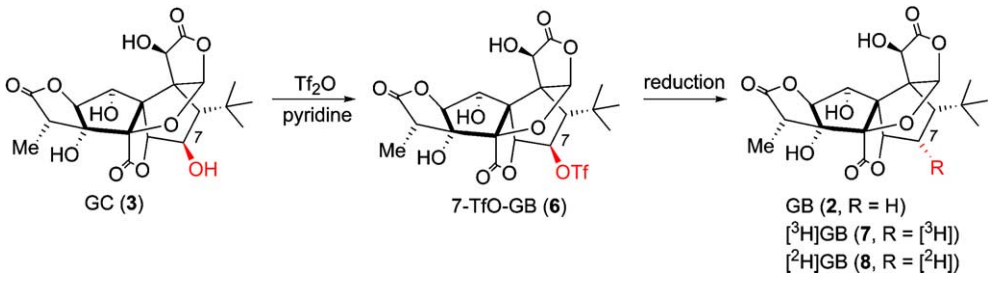
Among the five ginkgolides, GB (**2**) was selected for labeling, as GB (**2**) is both the most potent PAFR and glycine receptor antagonist.[2b] Moreover, it was envisaged that tritiated GB ([^{3}H]GB) could be prepared from GC in only two steps using site-selective reactivity of sulfonic anhydride, that is, trifluoromethanesulfonic anhydride, toward 7-OH of GC in a basic medium as originally reported by Teng.[15a] Thus the reaction of ginkgolide C (GC, **3**) with trifluoromethanesulfonic anhydride in pyridine gives 7-OTf-GB (**6**) in high yield (97%, Scheme 1).[15] This selectivity is noteworthy, as 10-OH, and in some cases 1-OH, of GC (**3**) is generally the more reactive hydroxyl group,[16] although we recently observed higher reactivity of 7-OH when acetylation is carried out under strong acidic conditions[17] (Scheme 2).



Scheme 2. Formation of a side product during reduction, due to the presence of base.

[^{3}H]GB (**7**) could then be prepared by treating 7-OTf-GB (**6**) with a tritiated reducing agent leading to introduction of the radiolabel in the 7-position of GB. Initially, the reaction was carried out with nonradioactive reducing agents; triflate **6** was reduced with tetrabutylammonium borohydride (nBu$_4$NBH$_4$) giving GB (**2**) in moderate yield. Several other reducing agents such as NaBH$_4$, NaBH$_3$CN, and LiBHEt$_3$ were investigated. However, in all cases when a 1:1 molar ratio of **6** and reducing agent was used, several side products were observed. Hence, we could not reproduce the results obtained by Teng.[15a] [^{3}H]GB (**7**) was then prepared by reaction of triflate **6** with [^{3}H]nBu$_4$NBH$_4$, the latter being prepared from [^{3}H]-sodium borohydride ([^{3}H]NaBH$_4$), and tetrabutylammonium chloride (nBu$_4$NCl) in [^{3}H]H$_2$O.[18] The specific activity of **7** was 3.8 Ci/mmol, which is very low considering the starting [^{3}H]NaBH$_4$ had a specific activity of 100 Ci/mmol. The low specific activity was presumably due to a rapid exchange between hydrogen in nonradioactive water contained in [^{3}H]H$_2$O and tritium in [^{3}H]NaBH$_4$. The [^{3}H]H$_2$O used in this study had a specific activity of 5 Ci/mL corresponding to only 0.09 Ci/mmol, which is the highest specific activity commercially available.

Instead [^{3}H]NaBH$_4$ was used as a reducing agent to provide [^{3}H]GB (**7**) with high specific activity. Although the initial studies showed that a 1:1 molar ratio of **6** and NaBH$_4$ did not give GB (**2**), a 1:5 molar ratio gave GB (**2**) in low to moderate yields, thus providing a



Scheme 1. Preparation of labeled ginkgolide B.

potential means of getting [^{3}H]GB (**7**) with high specific activity. The incorporation was confirmed using $NaBD_4$ to give [^{2}H]GB (**8**), incorporation ca. 70%. However, when the reaction was carried out with [^{3}H]$NaBH_4$ in dry THF the desired [^{3}H]GB (**7**) was produced only in trace amounts. It was realized that the commercially available [^{3}H]$NaBH_4$ contained substantial amounts of NaOH, which in the presence of trace amount of water contained in the solvents could open the lactone rings. Moreover, a byproduct was observed; from a nonradioactive synthesis this product was shown to be 7-*epi*-GC (**9**), originating from a nucleophilic attack of the triflate group by NaOH.

These obstacles were solved by shortening the reaction time from 16 to 2 h, as well as by acidic work-up, thereby making sure that all lactone rings were closed. Under the revised reaction conditions [^{3}H]GB (**7**) was produced in 5.8% radiochemical yield (from [^{3}H]$NaBH_4$). The product was purified by preparative HPLC (retention time 9.6 min), by collection of fractions every 30 s. The radioactivity of the fractions was determined by scintillation counting aliquot (5 μL) of each fraction. The specific activity of [^{3}H]GB was 18 Ci/mmol, thus substantially higher than that of [^{3}H]GB prepared from [^{3}H]nBu$_4$NBH$_4$.

In conclusion, [^{3}H]GB has been synthesized with high specific radioactivity, thus providing an important tool for the studies of neuromodulatory properties of ginkgolides.

Acknowledgements

Financial supports from NIH grants MH 68817 (K.N.), MH 6336 (M.S.) and a postdoctoral fellowship from the Alfred Benzon Foundation (K.S.) are gratefully acknowledged.

References and notes

1. Drieu, K.; Jaggy, H. In *Ginkgo biloba—Medicinal and Aromatic Plants—Industrial Profiles*; van Beek, T. A., Ed.; Harwood: Amsterdam, 2000; Vol. 12, pp 267–277.
2. (a) Hasler, A. In *Ginkgo biloba—Medicinal and Aromatic Plants—Industrial Profiles*; van Beek, T. A., Ed.; Harwood: Amsterdam, 2000; Vol. 12, pp 109–142; (b) Strømgaard, K.; Nakanishi, K. *Angew. Chem., Int. Ed.* **2004**, *43*, 1640–1658.
3. DeFeudis, F. V.; Drieu, K. *Curr. Drug Targets* **2000**, *1*, 25–58.
4. (a) Logani, S.; Chen, M. C.; Tran, T.; Le, T.; Raffa, R. B. *Life Sci.* **2000**, *67*, 1389–1396; (b) Oken, B. S.; Storzbach, D. M.; Kaye, J. A. *Arch. Neurol.* **1998**, *55*, 1409–1415; (c) Kleijnen, J.; Knipschild, P. *Lancet* **1992**, *340*, 1136–1139; (d) van Dongen, M. C. J. M.; van Rossum, E.; Knipschild, P. In *Ginkgo biloba—Medicinal and Aromatic Plants—Industrial Profiles*; van Beek, T. A., Ed.; Harwood: Amsterdam, 2000; Vol. 12, pp 385–442.
5. (a) Polich, J.; Gloria, R. *Hum. Psychopharmacol. Clin. Exp.* **2001**, *16*, 409–416; (b) Rigney, U.; Kimber, S.; Hindmarch, I. *Phytother. Res.* **1999**, *13*, 408–415; (c) Kennedy, D. O.; Scholey, A. B.; Wesnes, K. A. *Psychopharmacology* **2000**, *151*, 416–423; (d) Stough, C.; Clarke, J.; Lloyd, J.; Nathan, P. J. *Int. J. Neuropsychopharmacol.* **2001**, *4*, 131–134.
6. (a) Krieglstein, J.; Ausmeier, F.; El-Abhar, H.; Lippert, K.; Welch, M.; Rupella, K.; Henrich-Noack, P. *Eur. J. Pharm. Sci.* **1995**, *3*, 39–48; (b) Rapin, J. R.; Zaibi, M.; Drieu, K. *Drug Dev. Res.* **1998**, *45*, 23–29.
7. (a) Ishii, S.; Shimizu, T. *Prog. Lipid Res.* **2000**, *39*, 41–82; (b) Prescott, S. M.; Zimmerman, G. A.; Stafforni, D. M.; McIntyre, T. M. *Annu. Rev. Biochem.* **2000**, *69*, 419–445; (c) Maclennan, K. M.; Smith, P. F.; Darlington, C. L. *Prog. Neurobiol.* **1996**, *50*, 585–596.
8. Singh, M.; Saraf, M. K. *Drugs Future* **2001**, *26*, 883–888.
9. (a) Kato, K.; Clark, G. D.; Bazan, N. G.; Zorumski, C. F. *Nature* **1994**, *367*, 175–179; (b) Kobayashi, K.; Ishii, S.; Kume, K.; Takahashi, T.; Shimizu, T.; Manabe, T. *Eur. J. Neurosci.* **1999**, *11*, 1313–1316; (c) Chen, C.; Magee, J. C.; Marcheselli, V.; Hardy, M.; Bazan, N. G. *J. Neurophysiol.* **2001**, *85*, 384–390.
10. (a) Smith, P. F.; Maclennan, K. M.; Darlington, C. L. *J. Ethnopharmacol.* **1996**, *50*, 131–139; (b) Smith, P. F.; Maclennan, K. M. *Curr. Opin. Anti-Inflamm. Immunol. Invest. Drugs* **1999**, *1*, 205–218.
11. (a) Kondratskaya, E. L.; Lishko, P. V.; Chatterjee, S. S.; Krishtal, O. A. *Neurochem. Int.* **2002**, *40*, 647–653; (b) Ivic, L.; Sands, T. T. J.; Fishkin, N.; Nakanishi, K.; Kriegstein, A. R.; Strømgaard, K. *J. Biol. Chem.* **2003**, *278*, 49279–49285.
12. Moreau, J.-P.; Eck, J.; McCabe, J.; Skinner, S. *Presse Méd.* **1986**, *15*, 1458–1463.
13. Weinges, K.; Schick, H.; Irngartinger, H.; Oeser, T. *Tetrahedron* **2000**, *56*, 3173–3176.
14. Suehiro, M.; Simpson, N. R.; van Heertum, R. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 485–491.
15. (a) Teng, B.-P. U.S. Patent 5,599,950, 1997 (*Chem. Abstr.* **1996**, *124*, 117659s, *Chem. Abstr.* **1996**, *124*, 176580g); (b) Vogensen, S. B.; Strømgaard, K.; Shindou, H.; Jaracz, S.; Suehiro, M.; Ishii, S.; Shimizu, T.; Nakanishi, K. *J. Med. Chem.* **2003**, *46*, 601–608.
16. (a) Corey, E. J.; Rao, K. S.; Ghosh, A. K. *Tetrahedron Lett.* **1992**, *33*, 6955–6958; (b) Weinges, K.; Schick, H. *Liebigs Ann. Chem.* **1991**, 81–83; (c) Strømgaard, K.; Saito, D. R.; Shindou, H.; Ishii, S.; Shimizu, T.; Nakanishi, K. *J. Med. Chem.* **2002**, *45*, 4038–4046.
17. Jaracz, S.; Strømgaard, K.; Nakanishi, K. *J. Org. Chem.* **2002**, *67*, 4623–4626.
18. For preparation of nBu$_4$BD$_4$: Cazelles, J.; Robert, A.; Meunier, B. *J. Org. Chem.* **1999**, *64*, 6776–6789.