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Terpene trilactones from *Gingko biloba*: From ancient times to the 21st century

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Abstract—Ginkgolides were always close to my heart. I continue to be strongly attracted to *Ginkgo biloba*, the ginkgolides and bilobalide. Starting in 1963, I became fascinated by these molecules while working on their isolation and structure elucidation in Sendai. Presumably, due to the ginkgolide studies, I received an invitation to join the faculty at Columbia University. After almost three decades of not touching the ginkgolide project, we have unexpectedly resumed the studies, at this time because of their enigmatic biological effects. This account is a reflection on earlier studies, as well as an outline of our current work.

1. *Ginkgo biloba* and its extract

The tree *Ginkgo biloba* or maidenhair tree (Fig. 1) was mentioned in the Chinese *Materia Medica* 5000 years ago.[1] *G. biloba* L. is the only surviving member of the Ginkgoaceae family and Ginkgoales order, which underscores its unique phylogenetic status. Fossil records show that the *Ginkgo* genus was present some 180 million years ago. The Ginkgoaceae peaked 130 million years ago with numerous widespread species, but gradually gave way to modern angiosperm seed plants. Today only one species, *G. biloba*, has survived. The morphology of the Ginkgo tree itself appears to have changed very little for over 100 million years and for this reason it is often called a 'living fossil'.

The Ginkgo takes its name from *ginkyo* in Japanese and *yinhsing* in Chinese; both words translate to 'silver apricot', referring to the appearance of the Ginkgo nuts. The Ginkgo tree can grow up to 40 m high, with stem diameters between 1 and 4 m, and can reach ages of more than 1000 years. Vertical growth generally slows down with the onset of sexual maturity at around 25 years. The Ginkgo tree can persist under conditions of low light and nutrient scarcity and is highly resistant to bacteria, fungi, and viruses. Furthermore, its resistance to air pollution has made *G. biloba* a popular roadside tree in urban areas of Japan, Europe, and Northern America.

The Ginkgo tree is deciduous, having separate male and female trees with distinct sex chromosomes XY and XX. The green, leathery, fan-shaped leaves turn golden-yellow in autumn. The male leaves are divided into two distinct lobes, hence, the notation *biloba*, while female leaves are not divided (Fig. 2).

Figure 1. Ginkgo and Riverside Church, New York (left); Fall Ginkgo, Tohoku University, Sendai campus.

Keywords: *Ginkgo biloba*; Terpene trilactone; Ginkgolide; Bilobalide; Natural products.
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Figure 2. Female ginkgo leaves and nuts.

Along the Columbia River Plateau is found the Ginkgo Petrified Forest Park in the State of Washington. A remarkable feature of this park is that a variety of trees and plants are represented. The tree had been cultivated in China for several thousand years, and according to some earliest references dating back to the early 11th century Song dynasty, it was appreciated for its beauty and its edible nuts. Today the Gingko tree is cultivated partly to meet demands for its nuts, a delicacy in Chinese and Japanese cuisines. Raw Ginkgo nuts contain the toxin 4-*O*-methylpyridoxine, which can result in serious food poisoning. The tree was introduced into Japan from China in the 12th century, and some 500 years later to Europe and North America. The use of *G. biloba* for medicinal purposes was first mentioned in 1505 AD in a book by Liu Wan-Tai. In the Chinese *Materia Medica* 'Pen Tsao Ching' from 1578, *G. biloba* is described as a treatment for senility in aging members of the royal court. In these old records, it is mainly the use of nuts that is described. The term 'Ginkgo' was first used by the German physician and botanist Engelbert Kaempfer in 1712, but Linnaeus provided the terminology '*Ginkgo biloba*' in 1771.

G. biloba has generated immense interest for its reputed value in treatment of memory-related afflictions.[2] Over 7 billion dollars are spent annually on botanical medicines, and *Ginkgo biloba* ranks first among herbal medications. Fifty million *Ginkgo biloba* trees are grown, especially in China, France, and South Carolina, USA, producing over 8000 t of dried leaves each year to address the commercial demand for *Ginkgo biloba* products.[3]

EGb 761,[4] a standardized extract of the leaves, contains 5–7% ginkgolides[5–8] and bilobalide (BB),[9] collectively called terpene trilactones, TTLs (Fig. 3), along with 22–24% of flavonoids, for example, quercetin, kaempferol, and isohamnetin, and less than 5 ppm ginkgolic acids (also known as anacardic acids).[4] This limit was imposed because alkylphenols, such as ginkgolic acids, have been reported to induce contact dermatitis;[10] cytotoxicity was also reported for these compounds.[11]

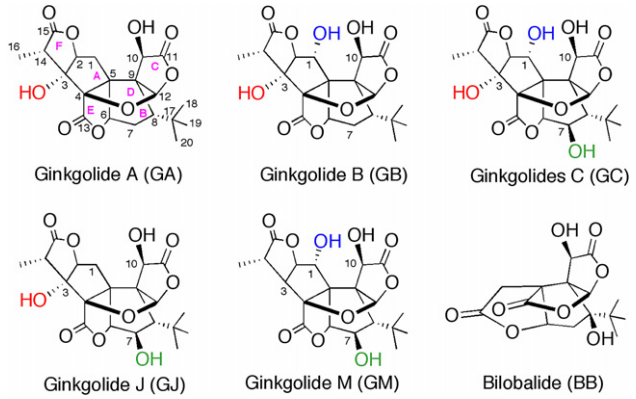


Figure 3. Ginkgolides and bilobalide.

Although recent research has focused on the medicinal value of the leaves,[12] almost nothing is known in terms of the molecular structural basis of the mode of action of the *Ginkgo biloba* extract. The extracts were reported to behave as an anti-asthmatic,[13] scavenge radicals,[14] reduce cerebral insufficiency,[15] alleviate the symptoms of mild to moderate Alzheimer-type dementia, and improve short-term memory in healthy subjects.[16,17] While numerous investigations have attempted to determine the effects of the individual constituents of *Ginkgo biloba* extracts on the central nervous system,[18] this aspect is still not well understood. Studies involving TTLs might provide the answers. The finding that ginkgolide B (GB, BN 52021), a minor component of EGb 761, is a potent inhibitor of the platelet-activating factor receptor (PAFR) is quite significant and has led to a dramatic interest in studies on *G. biloba*.[19,20] While platelet-activating factor receptor (PAF) has been linked to long-term potentiation (LTP)[21] and an increase of intracellular Ca^{2+},[22] the role of PAF in the CNS is not fully understood. BB, the predominant TTL present in the leaves, has been reported to strongly inhibit phospholipase 2 (PLA_2), whose activation initiates a cascade leading to neuronal death, activity in the brain, and may contribute to the anti-ischaemic activity of EGb 761.[23]

Among numerous effects displayed by EGb 761 in the central nervous system,[18] those related to AD,[24] dementia, and memory[25–27] are of particular importance. AD represents the most common form of dementia among those over 65 years of age and is now the third most expensive health care problem in the US, exceeded only by cancer and cardiovascular disease. AD currently affects ca. 4 million Americans and imposes an annual economic burden estimated between $80 and $100 billion. There is rising interest in the development of pharmacological agents, not only for the treatment of AD, targeting numerous neuroreceptors,[28] but also for the much more common, mild cognitive decline associated with normal, nondisease aging, defined as 'age-associated memory impairment' (AAMI). There is evidence suggesting that the patients with AAMI have an increased risk of developing dementia.[29]

EGb 761 is among the most prescribed medications in Germany and France,[25] and is recommended more often

than the calcium channel blocker Nimotop (nimodipine) for treatment of dementia.[30] In some countries EGb 761 is marketed under trade names such as Tanamin® (Korea), Tanakan® (France), Rökan® (Germany), and BioGinkgo®(USA) for the treatment of "...disturbed performance in organic brain syndrome within the regimen of a therapeutic concept in cases of demential syndromes with the following principal symptoms: memory deficits, disturbances in concentration, depressive emotional condition, dizziness, tinnitus, and headache. The primary target groups are dementia syndromes, including primary degenerative dementia, vascular dementia, and mixed forms of both."[31]

In two pivotal studies, a total of 549 AD patients were evaluated for the effect of EGb 761.[28,26] In both studies, the extract significantly slowed the loss of cognitive symptoms of dementia, and regression on certain data points was delayed by 7.8 months. An indirect comparison with similar data for the currently available AD treatments, Aricept® (donezepil, 9.5 months) and Exelon® (rivastigmine, 5.5 months), both acetylcholinesterase inhibitors, placed EGb 761 between these two drugs. Aside from the above-mentioned clinical trials, many clinical studies have been performed with extracts of *G. biloba*,[2] and virtually all trials report positive results regarding various aspects of cerebral insufficiency. Interestingly, Beaufour Ipsen (France) and Yuyu Industrial Co. (Korea) are developing EGb 761 in advanced clinical trials for treatment of mild cognitive impairment and neurodegenerative diseases.[32] Recent studies on healthy adults using a computerized test battery have shown beneficial effects of *G. biloba* extract on the short-term working memory,[17] and similar results were reported in two other recent studies.[16,33]

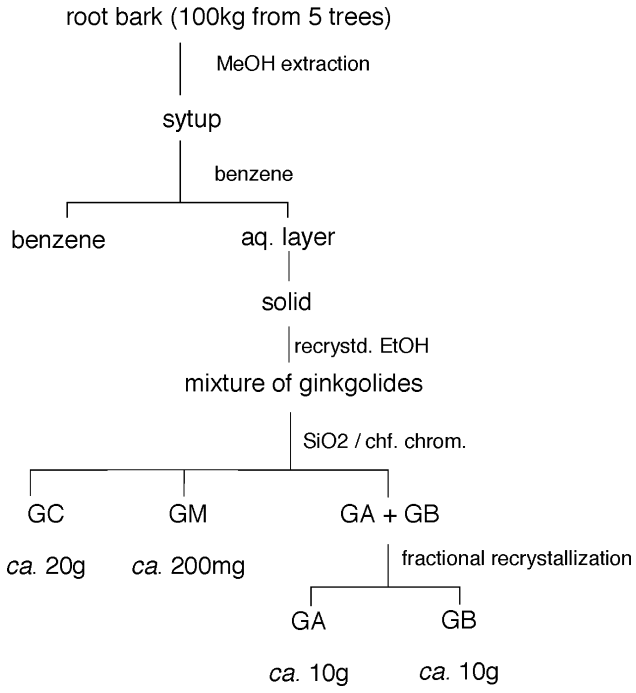
Although numerous studies have been carried out, the specific effects of EGb 761 are not well understood. Several suggestions have been made; for example, changes in brain function may be associated with the prevention of age-related decreases in 5-HT binding[34] or α_2-adrenergic receptor density in cerebral membranes.[35] Recently, it was found that EGb 761 modulated the expression of several genes, including genes believed to be involved in AD.[36]

2. Outline of early chemical studies

I moved to Tohoku University, Sendai, from what now is Tsukuba University (previously Tokyo Kyoiku University) in spring 1963 to succeed Professor S. Fujise and started working on the isolation of the ginkgolides, which Fujise had started in 1960. In 1964, we received permission to fell five typhoon-damaged Ginkgo trees; 100 kg of the root bark (Fig. 4) gave after extraction, chromatography, and a tedious 10- to 15-step fractional recrystallization about 10 g of GA, 10 g of GB, 20 g of GC, and 200 mg of GM (Scheme 1).

The isolation assay for ginkgolides was quite simple: follow the bitter taste with our tongues. However, purification of ginkgolides was seriously hampered by their

Figure 4. Group members and helpers processing root barks at the corner of the Chemistry Department, Tohoku University, around 1965. My office was on the second floor corner.



Scheme 1. Original extraction scheme.

remarkable tendencies to exhibit polymorphism and form mixed crystals. These were pre-HPLC days, when 100 MHz (or 100 Me) NMR was the most powerful and high resolution MS was not common; carbon NMR was unheard of. We all derived great fun through the intense day and night research, and for me it was the last classical and romantic structural study. Numerous chemical reactions were performed and structural elucidation of these multioxygenated cage molecules was accompanied by surprises and excitement, as if unfolding a mystery. Such an experience is seldom encountered these days due to the unromantic developments in isolation and especially spectroscopic techniques. The intensive work of the ginkgolides team was rewarded with excitement when the ginkgolides' structures rapidly unfolded in September

1966 after the finding of many extraordinary reactions (about 50 derivatives were prepared and submitted to NMR measurements by Maruyama and Terahara, 1967), as well as the first encounter with the NMR intramolecular nuclear Overhauser effect (NOE), unknown at that time. Although no special biological activity was found for these molecules at that time, hence, the tongue assay, it is extraordinary that in 1985 the ginkgolides were found to be potent and selective antagonists of PAF; a host of additional activities have since been found.

The most unique constituents of *G. biloba* are the TTLs, that is, ginkgolides and BB. Ginkgolides A, B, C, and M were isolated from the root bark of *G. biloba* in 1967, and their complex structures deduced. A few years later, a related structure, BB, was established,[9] and in 1987 ginkgolide J was isolated from the leaves of *G. biloba*.[37] Interestingly, ginkgolide J was found only in the leaves, whereas ginkgolide M was found only in the root bark. Terpenoids have long been regarded to be biosynthesized through the mevalonate pathway, and a biosynthetic scheme based on incorporation of ^{14}C-labeled acetate, mevalonic acid, and methionine into ginkgolides by the cotton-wick method was forwarded.[38] However, using ^{13}C NMR as the major tool, Schwarz and Arigoni showed that ginkgolides were biosynthesized through an alternative path through the glyceraldehyde–pyruvate pathway (Scheme 2).[39]

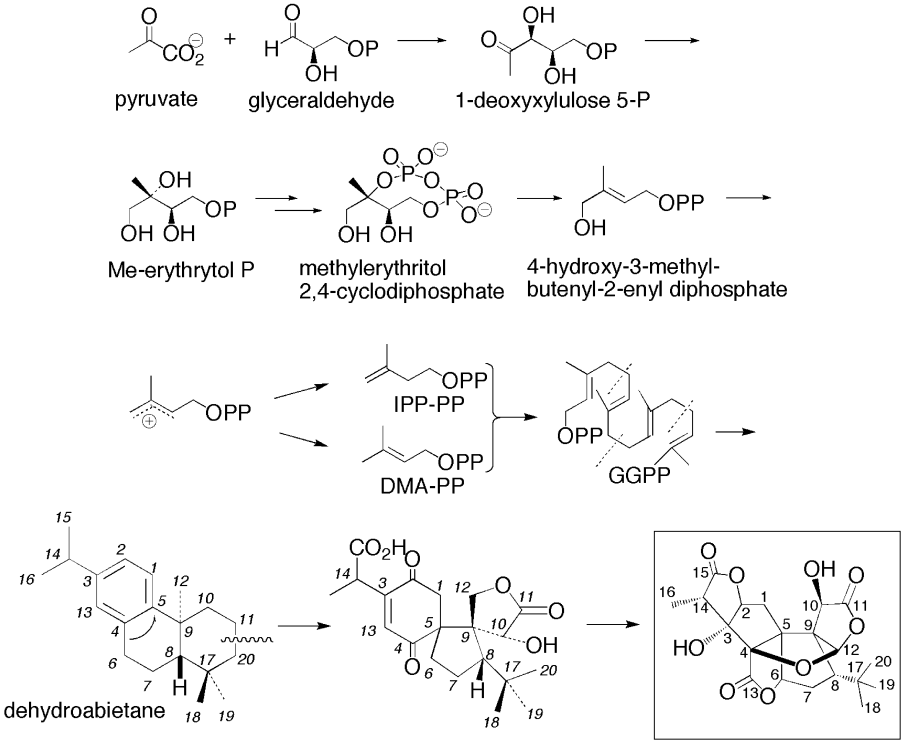
Independently, Rohmer also showed the surprising existence of a similar nonmevalonate route in the ancient hopanoids.[40] Moreover, the sites of mevalonate and nonmevalonate pathways appear to be compartmentalized, even in the same plant. The nonmevalonate biosynthetic routes now appear to be quite common. Recently, a ginkgolide biosynthetic gene was cloned and the in vitro observation of an isolated ginkgolide biosynthetic enzyme was reported.[41]

Due to their structural complexity, TTLS have attracted great interest as targets for total syntheses. The group of E.J. Corey first synthesized BB,[42] and shortly afterward they accomplished the total syntheses of ginkgolide A[43] and ginkgolide B.[44] Crimmins and co-workers have recently completed a new synthesis of ginkgolide B.[45] See Ref. 46 for a review describing our more recent studies.

The rapidly increasing interest in *G. biloba* studies is clear from the bar graph (Fig. 5) that shows the number of publications on *Ginkgo* and trilactones. The slight increase in the late 1960s reflects our structural studies, while the conspicuous increase after the mid 1980s is due to the finding that TTLs are PAFR antagonists. However, very little is known regarding which components of EGb 761 are efficacious; the molecular basis for the action of *G. biloba* constituents on the CNS and memory is hardly understood. Partially, this is because the neuroprotective action of EGb 761 and its active components is extremely complex, and it is known to affect a variety of biological pathways.[47]

3. Ginkgolide structure determination by classical methods, including IR and ORD

Ginkgolides are diterpenes with an aesthetically beautiful cage skeleton consisting of six 5-membered rings,



Scheme 2. The terpenoid nonmevalonate biosynthetic pathway.

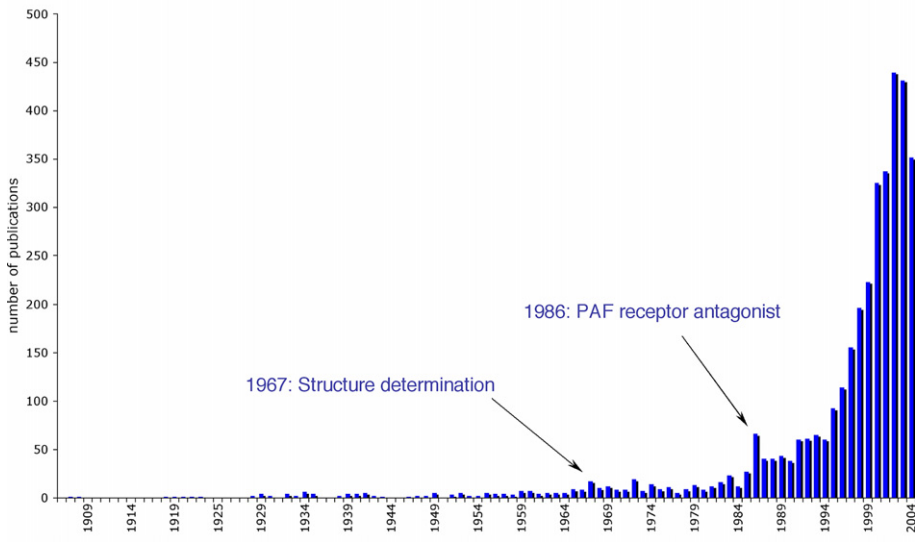


Figure 5. Number of publications with words *G. biloba* and ginkgolides in abstract/titles. CAPLUS database, Scifmder Scholar ACS, March 2005.

that is, a spiro[4.4]nonane carbocyclic ring, three lactones, and a tetrahydrofuran (Fig. 3). The sesquiterpenoid bilobalide cage structure was determined in 1971 in collaboration with the groups of Professors Major and Bähr at Virginia and Heidelberg, respectively.[9]

All ginkgolides are bitter tasting solids that have no melting point. They are extremely stable towards acids: evaporation to dryness of a ginkgolide nitric acid solution results in recovery of starting material. The molecular composition of ginkgolides (GA $C_{20}H_{24}O_9$; GB $C_{20}H_{24}O_{10}$; GC $C_{20}H_{24}O_{11}$; GM $C_{20}H_{24}O_{10}$) was determined by elemental analysis. The molecular formula of a relatively volatile GA dimethyl ether was determined by HRMS calcd for $C_{22}H_{82}O_9$, 436.173; found: 436.168.

3.1. *tert*-Butyl group

Although the NMR (all spectra measured in TFA for solubility reasons) always showed a 9-proton singlet at 1.2–1.3 ppm, the presence of the *t*-Bu group was established only after detection of a strong *t*-Bu peak in the HRMS at 57.074 (calcd 57.070) and isolation of pivalic acid or *t*-Bu carboxylic acid, characterized as its crystalline *p*-bromophenacyl ester, obtained by chromic acid oxidation, or Kuhn Roth oxidation, of ginkgolide C. *G. biloba*. TTLs are probably still the only terrestrial terpenoid carrying a *t*-Bu group (*t*-Bu groups are present in several marine compounds).

3.2. Hydroxyl groups

The number and nature of hydroxyls, namely, *sec*- and *tert*-OH groups, were determined by measurement of NMR in DMSO-d_6, before and after D_2O addition.

3.3. Carbonyl functions

The single ill-defined IR (KBr) peak at 1780 cm^{-1} was assigned as lactonic. Namely, it is replaced by carboxylate bands at 1540 and 1400 cm^{-1} in Na salts prepared by evaporating an aq NaOH solution. Conventional titration opened only two lactones (lactones F and C); however, when a solution of the ginkgolides in 1.0 N aq NaOH was evaporated to dryness in vacuo at 50 °C (IR KBr showed all lactones had been cleaved) and subsequently redissolved in water and titrated with 1.0 N HCl, GA, GB, and GCE all consumed ca. 2.5 moles of alkali. Namely, lactone E was opened under highly basic conditions, that is, evaporation of NaOH solution, and once cleaved it remained open upon dissolution in water. The absence of a ketone group was also suggested by the plain negative ORD (optical rotatory dispersion curve; circular dichroic spectrometers were not yet available) that exhibited no Cotton effect (CE) in the 250–270 nm range.

The first indication for three lactones came from analysis of so-called 'GA triether', which was corroborated by an 'exhaustive' lactone titration. Because the connectivity of proton groups in the ginkgolide structures is blocked by the intervening quaternary carbons, the ginkgolide NMR are overly simple so that they do not impart much information (Fig. 6).

Without giving much thought to the outcome, GA was reduced with $LiAlH_4$ to see what would happen; not surprisingly this resulted in a seemingly useless syrup, an octaol. However, drying the syrup, which must have contained a trace of acid in an oven, gave a solid that had sublimed to the oven ceiling; this became the critical compound for structural studies, that is, 'GA triether' (because of ring D, it is in fact a tetraether). Further amounts of the crystals were made by heating the powder in vacuum at 150 °C for 3 h.

A detailed study of the NMR of GA triether by Vyn Woods and Iwao Miura led to the observation of what is now called NOE, which at that time was unknown[48]. To analyze the complex triether spectrum, a thorough decoupling of the *t*-Bu group was performed. They noticed that irradiation of the *t*-Bu peak led to 10–33%

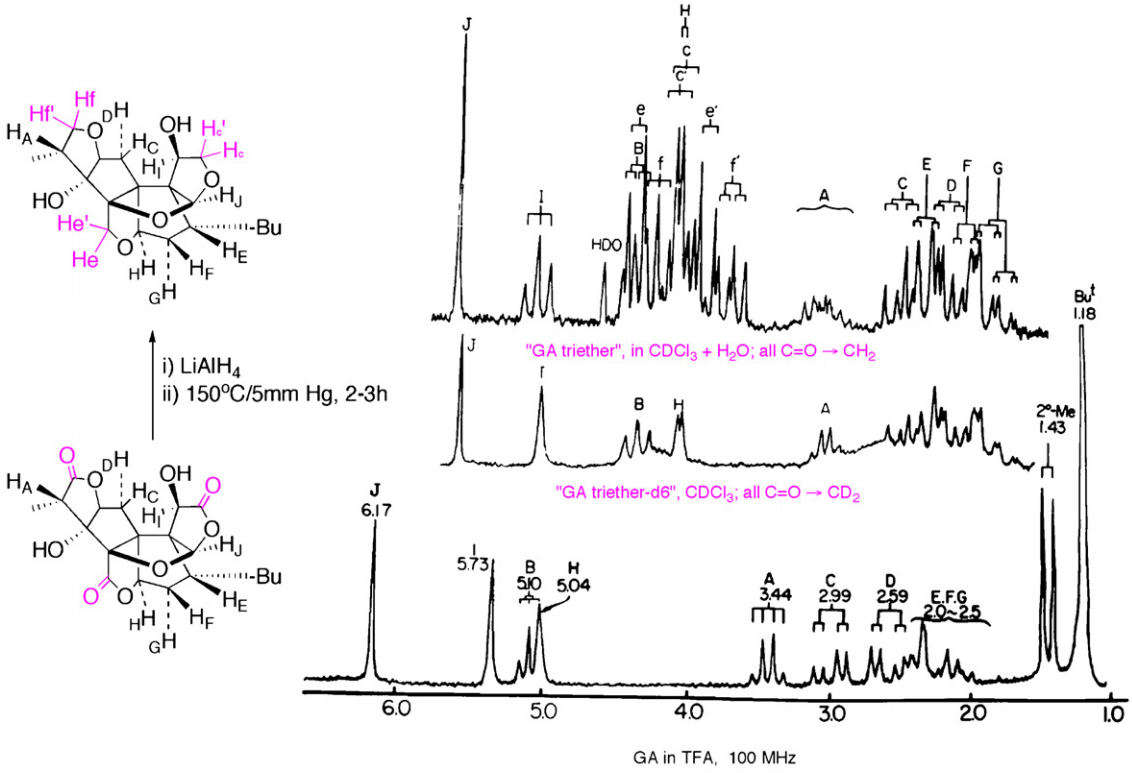


Figure 6. NMR of GA, its triether and deuterated analog. Newly introduced hysrogens are shown by small case letters.

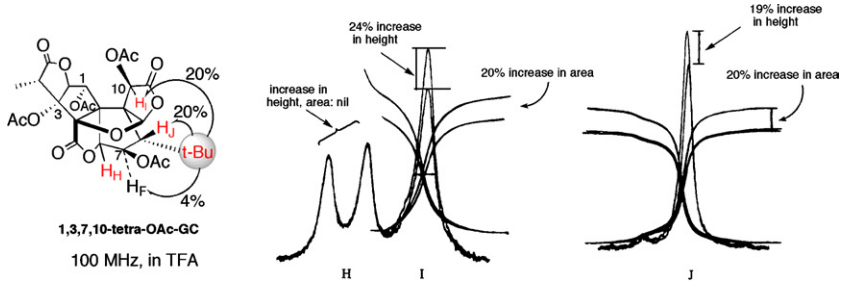


Figure 7. Intramolecular nuclear Overhauser effects observed by Woods and Miura.

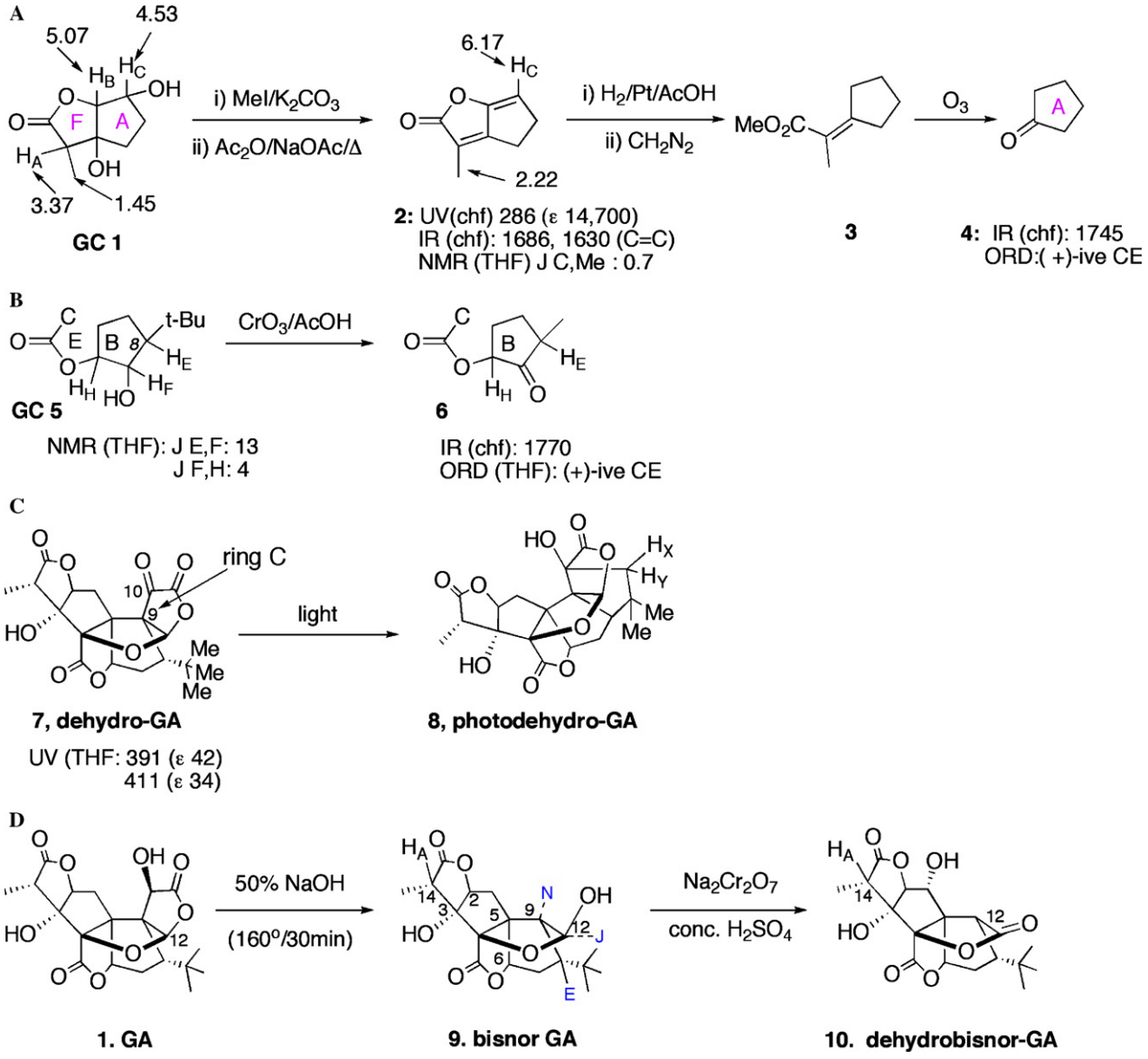
enhancements in the area of certain proton signals (Fig. 7).

Instead of dismissing this as an integrator error, they repeated the measurements until they were convinced that the area increases were true and thought that it may be related to the Overhauser effect that was known in electron spin resonance spectroscopy (1966). Shortly after he had interpreted the results correctly, the first NOE paper by Anet and Bourn dealing with dimethylacrylic acid and another cage molecule appeared (1965). Vyn's uncompromising attitude had led to the finding of NOE. However, we were not fully aware of the power of NOE and the title of our first paper dealing with NOE was quite modest:[48] 'The ginkgolides. V. Some aspects of their NMR spectra.' As depicted in Figure 6, irradiation of the *t*-Bu group in GC tetraacetate enhances the integrated area of protons I and J by 20%; however, for proton H there is no increase.

A comparison of the complex triether and simple GA spectra shows that except for peaks designated by small case letters cc′, ee′, and ff′, the two are quite similar; namely, the three doublets of doublet methylene peaks were introduced by $LiAlH_4$ reduction. This was confirmed by the 'GA triether-d_6' spectrum resulting from LiAlD4 reduction in which all peaks denoted by small case letters have disappeared. Moreover, similarity in the GA and triether-d_6 NMR shows that the original ring structure is retained despite the difference between three lactones and three ether rings.

3.4. Ether linkage

Establishment of the number of OH and lactone groups leaves one more oxygen function to be accounted for. This is assigned to an ether bond. In classical structure determinations, unaccounted oxygen functions are assigned to ethers (Scheme 3).



Scheme 3. Structure determination.

3.5. Rings F and A (Scheme 3a)

In **2**, peak H_c is W-coupled to the 2.22 ppm 14-Me peak. Ring F is lost upon hydrogenolysis of **2** to give **3**, the ozonolysis of which generates a five-membered ketone **4** (CE at 290 nm).

3.6. Ring B and protons E, F, and H (Scheme 3b)

Substitution patterns on ring B were based on J values of protons E, F and H. The IR of dehydro-GC **6** suggests a five-membered ketone: the high frequency for the five-membered ketone was attributed to the ring strain originating in the attachment of the *t*-Bu at C-8.

3.7. Ring C is an α-hydroxylactone (Scheme 3c)

It was suspected that one of the lactones C is an α-hydroxylactone. If this is the case, oxidation should yield an oxo-lactone. Hence, GA was submitted to vigorous oxidation of GA with concd sulfuric acid and sodium bichromate expecting to get an oxolactone with a characteristic UV. In fact, this had occurred to give dehydro GA **7**, but the product **7** was for some time a source of confusion and misinformation. Namely, since **7** underwent facile photocyclization, even during TLC, to photodehydro GA **8** that has no UV, for a while it was thought that GA contained no α-hydroxylactone, thus leading to the proposal of a tentative wrong structure. The unusual spectroscopic and photochemical properties of **7** have been clarified.[49]

3.8. Linking of rings B and C (Scheme 3d)

Alkali fusion of GA under vigorous conditions simply resulted in the loss of two carbons as oxalic acid to give bisnor GA **9**. NMR showed that ring C was destroyed but other rings were intact. Furthermore, it was clear from decoupling that the newly formed proton N in **9**

was strongly coupled to proton E. Ring B is thus linked to hydroxylactone C by sharing C-9.

With respect to remaining hydrogen J, its low chemical shift at 6.2–6.3 ppm in ginkgolide and derivatives and the fact that it retains its singlet nature require it to be flanked by two oxygens. The only manner to do this is to place it on C-12 and link C-12 to the remaining ether oxygen.

Finally, the linking of *t*-Bu, GC part structure **1**, rings B/C, and ethereal oxygen in a systematic manner leads to eight structures; seven of these can be readily eliminated on the basis of NMR peak shifts in various derivatives and NOE, thus leading to the ginkgolides structure. Configurations of the hydroxyl groups were deduced by comparison of NMR data and straightforward chemical reactions.

4. Ginkgolides in the 21st century

4.1. Isolation of TTLs from *G. biloba* leaves or commercial extracts

Despite being the main active ingredients of the *G. biloba* extract, TTLs comprise only 6–7 wt %. Upon resumption of ginkgolide studies in 2000, chopping of typhoon-destroyed Ginkgo trees was no longer an option. In addition to developing an efficient extraction protocol from leaves, we were fortunate to receive generous supplies from Pharmanex of the extract BioGinkgo^{TM} that contains 22% flavonoids and 7% TTLs. A modification of a literature procedure,[50] yielded a simple way of quantifying the TTL content in the extract (Fig. 8).

The first facile extraction was accomplished by the German postdoc Dirk Lichblau. In this protocol, the leaves or BioGinkgo are boiled in water containing 5% H_2O_2, followed by extraction into ethyl acetate.[51] The key step was the use of the peroxide that destroyed the constituents leading to formation of stable emulsions during the subsequent extraction steps. Maintenance of pH 8-9 of the aqueous phase neutralized the acidic components in the mixture and led to efficient recovery of intact TTLs. Such procedures afforded a fraction containing ca. 60–70% TTLs in about 8 h.[51]

The ginkgolides are difficult to separate because of similar solubilities and chromatographic behavior. However, Dr. Stan Jaracz and Dr. Shahid Malik, applying a combination of benzylation, column chromatography, and debenzylation, developed a procedure suitable for a gram-scale isolation of individual ginkgolides and BB (Scheme 4).

Drs. Sergei Dzyuba and Hideki Ishii have since come up with an almost unprecedented short procedure to separate the TTL fraction from the whole crude extract. Namely, direct extraction of the solid powder of BioGinkgo with ethyl acetate selectively and quantitatively isolates all ginkgolides and BB, and nothing else, the yield of TTL being 7%, which is the entire TTL content.[52]

Other solvents such as THF, CH_3CN, and MeOH were inferior to EtOAc. They then attempted to use this procedure to isolate TTLs from *G. biloba* tree leaves, but failed: although BB and ginkgolides were observed (NMR) in the mixture, their complete separation from other components turned out to be problematic. Separation of TTL mixture into the individual TTLs can be accomplished by column chromatography, some prederivatizations, or even by old fashion crystallization.

Such unique preparation also brings up an interesting issue of how much we really know about the other components of the *G. biloba* extract. The fact that only TTLs are extracted into ethyl acetate might suggest that originally flavanoids are all or mostly glycosylated, and that sugars are cleaved during subsequent isolation steps giving rise to many flavanoids.

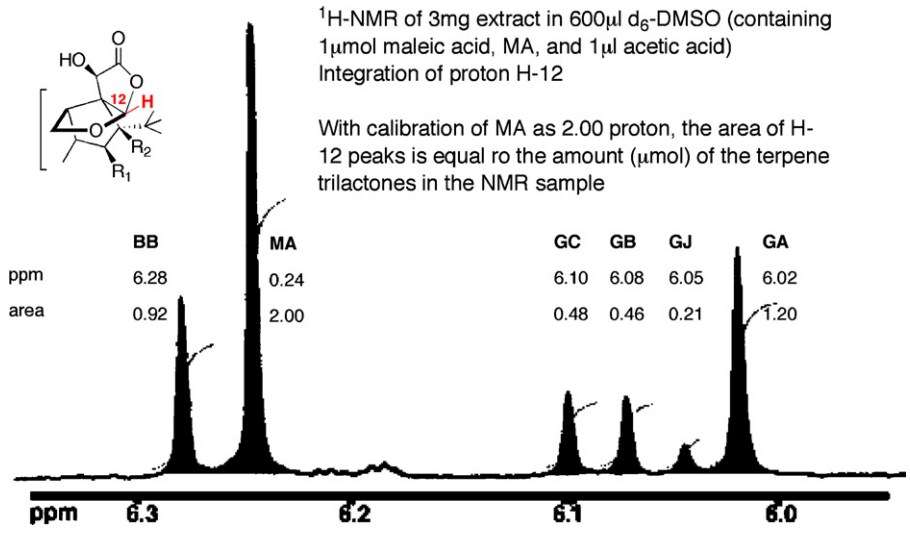
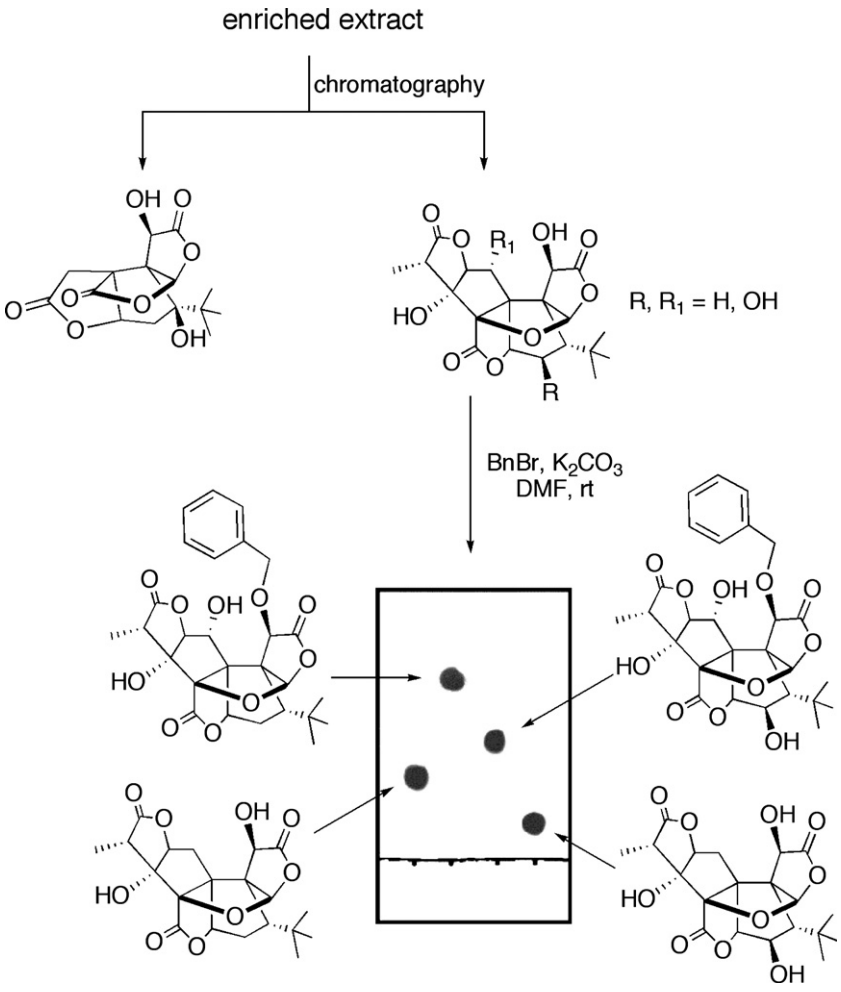


Figure 8. Quantification of ginkgolides by NMR.



Scheme 4. Multistep isolation of TTLs from the extract. TLC plate shows the separation of derivatized and nonderivatized finkgolides.

4.2. Core-modified ginkgolides

The main body of work on ginkgolides takes advantage of distinct reactivities of hydroxyl functionalities present on the ginkgolide skeleton. However, it is likely that the unique biological properties of ginkgolides arise from its cage-like structure. Modification of the cage structure or hydroxyl functions could lead to unexpected modes of biological interactions. The current chemical studies on ginkgolides also explore a novel paradigm of diversifying the structural pool of the ginkgolides—core-modified ginkgolides.

The example of such a modification was the GA triether, unexpectedly accomplished at Tohoku University. Further development along such lines was challenging and nontrivial. Despite the excellent synthetic hands of Hideki Ishii coupled with numerous modifications of reaction conditions, the efficient production of GA triether still led to sporadic results. Finally, Sergei and Hideki screened several reducing agents and found that GA triether can be reproducibly obtained in reasonable yields in just two steps (Scheme 5). Also, they were able to obtain a series of intermediate compounds that will be submitted to bioassays.

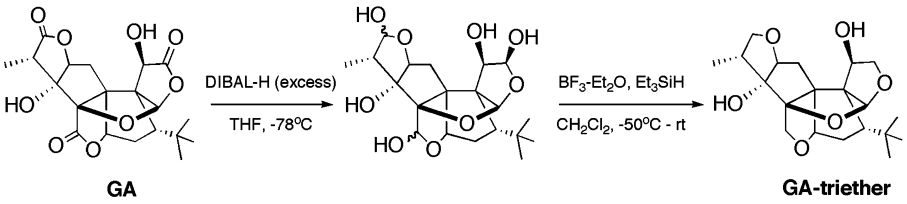
Unique reactivity of α-hydroxy lactone ginkgolide moiety toward $NaBH_4$ was discovered by Dr. Katsunori Tanaka and Professor Nina Berova.[53] Further developments explored the reactivity of α-hydroxy lactone moiety towards other nucleophilic reagents, such as Grignard and alkyl lithium reagents. Elaboration of this resulted in the preparation of lactols derivatives, which presented an attractive scaffold for further functionalization (Scheme 6).[54]

4.3. Interactions with glycine receptor

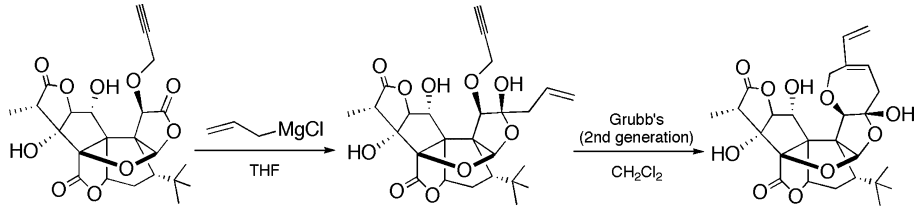
Glycine receptor (GlyR) is an anion-selective ligand-gated ion channel that mediates inhibitory synaptic transmission in the adult central nervous system.

We found that GC antagonizes glycine responses in the embryonic rat cortex in a use-dependent manner, and requires the presence of open channels in GLYR inhibition (Fig. 9).[55]

We then proceeded to SAR studies of GC. An enormous amount of work was performed by Stan Jaracz, graduate student at the time, and Stromgaard and co-workers,[56] who made over 40 ether-, ester-, and carbamoyl-derivatives of ginkgolide C leading to novel methods



Scheme 5. Twenty-first century preparation of GA triether.



Scheme 6. Extending the cage to a bowl.

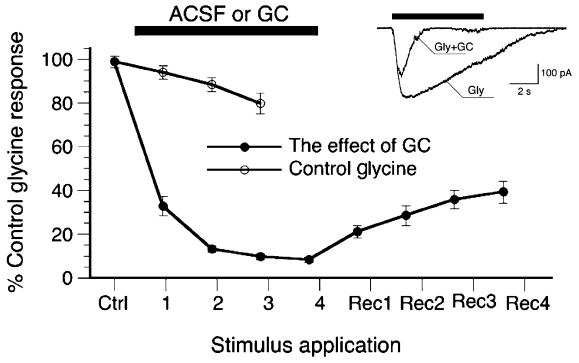


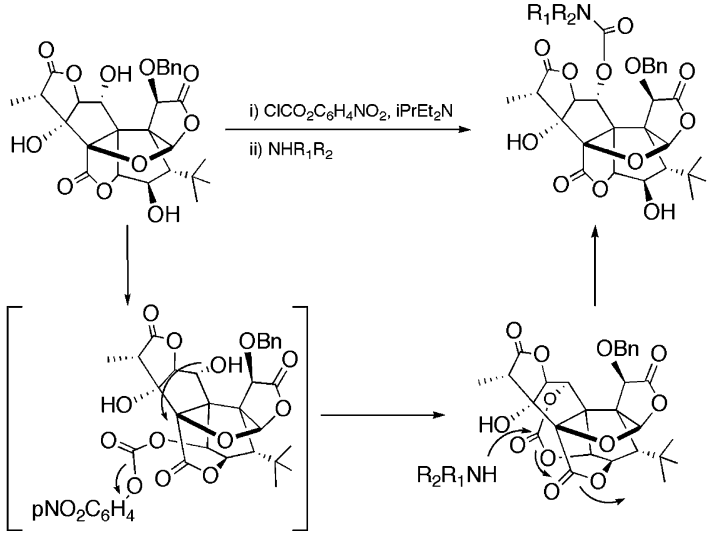
Figure 9. GlyR inhibition by GC.

for indirect functionalization at the 1-position (Scheme 7). However, all of these compounds were less potent than the native GC, leaving us with only one profound conclusion: hydroxy-groups are important for antagonistic activity.

4.4. Interactions with PAFR

In 1985, it was discovered that ginkgolide B was a potent antagonist of the PAFR, a 39 kDa G-protein coupled receptor.[19,57] This led to an extensive investigation into the clinical applications of ginkgolide B (BN 52021) as a PAFR antagonist by Beaufour Ipsen. The studies went to phase III clinical trials,[4] but like many other PAFR antagonists, ginkgolide B was never registered as a drug, primarily due to the lack of efficacy. These studies, however, showed that ginkgolide B was well-tolerated and showed very few, if any, side effects. The mechanism by which the PAFR and PAF (Fig. 10) are involved with the central nervous system is still unclear,[58] but there is no doubt that ginkgolides are worthy candidates to elucidate the physiological and pathological events associated with PAFR.

The findings that GB effects hippocampal LTP by inhibiting PAFR is the step in that direction.[59] PAF is



Scheme 7. Indirect functionalization of ginkgolide C derivatives.

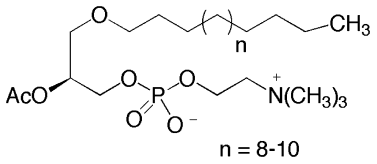


Figure 10. Structure of PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. McPAF is a methyl carbamyl analog of the acetate.

involved in several events in the central nervous system, including modulation of LTP, [21,60,61] increase of intracellular Ca^{2+},[22] and immediate early gene expression.[62–64] In LTP, PAF is believed to act as a retrograde messenger,[21] but using PAFR knock-out mice, different observations were made: Shimizu and co-workers showed that the PAFR is not required for LTP in the hippocampal CA1 region,[65] whereas Chen et al. showed that LTP was attenuated in hippocampal dentate gyrus neurons.[66] These discrepancies, however, may be explained by the different hippocampal areas observed.

Most of the studies on ginkgolide–PAFR interactions were done using radioactive [^{3}H] WEB 2086 displacement assays. In addition, Sonja Krane used a nonradioactive microphysiometry assay to evaluate the effects of TTLs on PAFR (Fig. 11).[67] Namely, inhibition of the response of PAF agonist McPAF caused by ginkolides was measured.

It is interesting that the presence or absence of one hydroxy-group at 7-position can convert GB, a potent PAFR antagonist (K_i 0.88 μM), into GC (K_i 12.6 μM) with low activity. Also fascinating is the fact that the stereochemistry at this position might be the most dominant factors: 7-*epi*-GC (7-α-OH) had a K_i value of 4.26 μM. In addition, various functionalities, such as halides, azides, and amines, exhibit various activities (Table 1).[68] Surprisingly, 7-chloro-containing ginkgolide derivative, 7α-Cl-GB, was shown to be the most potent nonaromatic ginkgolide derivative (K_i 0.11 μM).

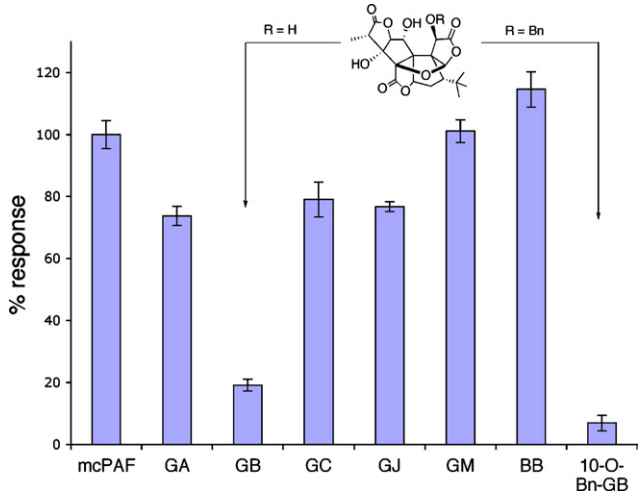
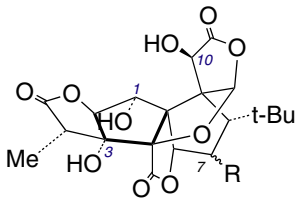


Figure 11. Inhibition of PAFR responses in the presence of ginkgolides.

Table 1. K_i values of GB, GC and 7-derivatives



Compound	R	K_i (μM)[a]
GB	H	0.88
GC	β-OH	12.6
7α-OAc-GB	α-OAc	7.84
7-eoi-GC	α-OH	4.26
7α-$OCOCH_2Ph$-GB	α-$OCOCH_2Ph$	2.40
7α-N_3-GB	α-N_3	0.55
7α-F-GB	α-F	0.99
7α-Cl-GB	α-Cl	0.11
7α-NHMe-GB	α-NHMe	0.61
7α-NHEt-GB	α-MHEt	1.62
7α-NH2-GB	α-NH2	8.64

[a] Inhibition of [^{3}H]-WEB 2086 binding. Values are means of two independent experiments performed in triplicate.

We intended to probe the ginkgolide–PAFR site-specific interactions through photolabeling experiments. Fortunately, introduction of photoaffinity labels, such as benzophenone, trifluoromethyldiazirine, and tetrafluorophenyl azide led to quite potent antagonists with K_i in the 0.09–0.15 μM range (Scheme 8).[69]

However, this was not the case for acetates, which were prepared as models for diazoacetates. Although a selective acetylation of ginkgolides was developed, all of the acetate derivatives were less potent than the native ginkgolides (Scheme 9).[70]

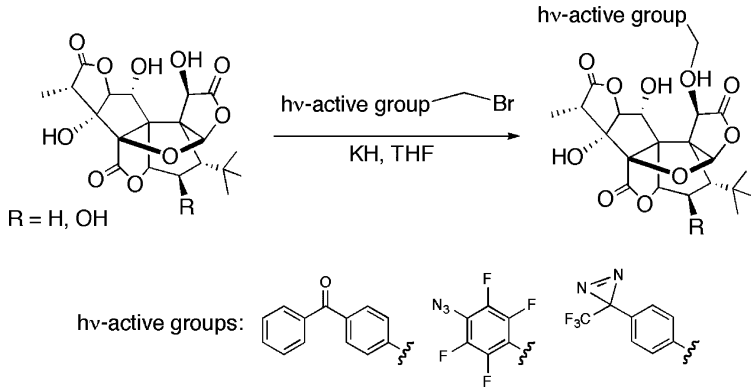
The numerous structure–activity studies (Fig. 12) performed so far suggest further vast opportunities for preparing more efficient compounds.

4.5. Relation to Alzheimer's disease (AD)

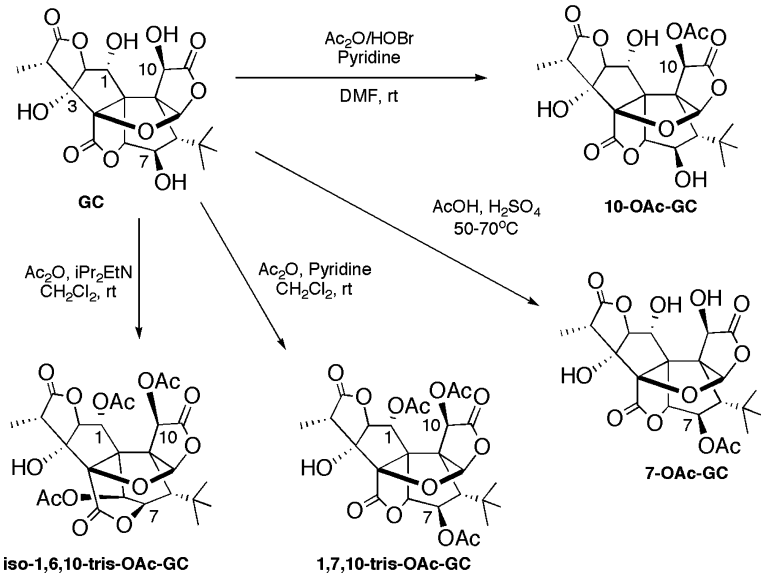
Ginkgolides have long been promoted as memory-enhancing natural products, and the work from this and other laboratories has provided some evidence that this is obviously the case. Recent interest in *G. biloba* extract as well as in ginkgolides has been driven by their inhibitory effects on the progression of AD.

In collaboration with the groups of Professors M. Shelanski and O. Arancio, College of Physicians and Surgeons, Columbia University, we were able to demonstrate that ginkgolides are indeed the components of the extract that inhibit or even eliminate the deadly effects of amyloid peptides on LTP. In a series of electrophysiological experiments, it was demonstrated that GA and GJ are two of the native ginkgolides that can mimic/replicate the effect of the TTL-enriched extract. Also, a synthetic derivative of GA, GA triether, was demonstrated to exhibit activity.

However, GJ is the least abundant ginkgolide in the TTL fraction, which collectively is only 6–7% in the



Scheme 8. Derivatization of GB with photoactivatable reagents.



Scheme 9. Acetylation of GC.

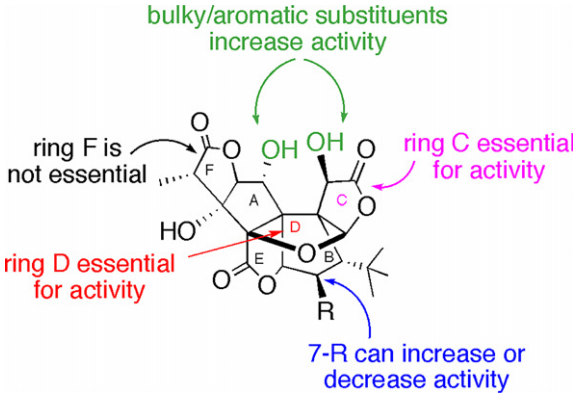


Figure 12. Ginkgolide–PAFR structure–activity relationship.

whole *G. biloba* extract. In order to secure large quantities of GJ for further biological and behavioral animal studies, kilogram quantities of the extract will be required, which despite our recently developed efficient isolation procedures still presents a major problem. We have therefore started to explore synthetic methods to convert the other more abundant ginkgolides, for example, GC, into GJ. Such procedures may also present an entry to new ginkgolide derivatives.

We have also addressed the issue of whether the ginkgolides are capable of directly interacting with amyloid peptides. This is quite an intriguing paradigm, since for all these years our aim was to investigate the interaction of ginkgolides, with large biomolecular receptors of several dozen kDa MW. The current work, in contrast, is to study interactions between the ginkgolides and small molecular peptides. Amyloid peptides are particularly prompt to aggregate starting from the monomeric species and proceed to undergo a self-assembly process via formation of low and high molecular weight oligomers, eventually resulting in the formation of protofibrils and fibrils. The ginkgolides have several potential avenues for interaction by either affecting monomer to oligomer

equilibrium or modulating the assembly of already preformed amyloid oligomers. These studies performed in collaboration with Professor David Lynn at Emory University are opening new possibilities of exploring the relationship of ginkgolides to AD. Preliminary results are clearly showing that ginkgolides are beneficial for memory improvement and dementia treatment. Our goal is directed towards clarifying the beneficial basis of the *G. biloba* dietary supplement.

5. Concluding remarks

The studies on the ginkgolides that were inherited from the late Professor S. Fujise upon my appointment as his successor in 1963 are quite unexpectedly developing into a worthwhile project that necessitates collaborative studies among various science, bioscience, biomedical, and biophysical disciplines.

Acknowledgments

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References and notes

1. Deng, Q. *Drug Use Perspect.* **1988**, *1*, 57–58.
2. *Ginkgo biloba*; van Beek, T. A. E., Ed.; Harwood Academic Publishers: Amsterdam, 2000.
3. Schmid, W. *Nature* **1997**, *386*, 755.
4. Drieu, K.; Jaggy, H. Medicinal and Aromatic Plants—Industrial Profiles In *Ginkgo biloba*; van Beek, T. A., Ed.; Harwood Academic: Amsterdam, 2000; Vol. 12, pp 267–277.
5. Maruyama, M.; Terahara, A.; Itagaki, Y.; Nakanishi, K. *Tetrahedron Lett.* **1967**, 299–303.
6. Maruyama, M.; Terahara, A.; Itagaki, Y.; Nakanishi, K. *Tetrahedron Lett.* **1967**, *4*, 303–308.
7. Maruyama, M.; Terahara, A.; Nakadaira, Y.; Woods, M. C.; Nakanishi, K. *Tetrahedron Lett.* **1967**, *4*, 309–313.
8. Nakanishi, K. *Pure Appl. Chem.* **1967**, *14*, 89–113.
9. Nakanishi, K.; Habaguchi, K.; Nakadaira, Y.; Woods, M. C.; Maruyama, M.; Major, R. T.; Alauddin, M.; Patel, A. R.; Weinges, K.; Bähr, W. *J. Am. Chem. Soc.* **1971**, *93*, 3544.
10. Leppoittevin, J. P.; Benezra, C.; Asakawa, Y. *Arch. Dermatol. Res.* **1989**, *281*, 227–230.
11. Siegers, C. P. *Phytomedicine* **1999**, *6*, 281–283.
12. Del Tredici, P. In *The Evolution, Ecology and Cultivation of Ginkgo biloba*; Harwood Academic: Amsterdam, 2000; Vol. 12.
13. Mahmoud, F.; Abul, H.; Onadeko, B.; Khadadah, M.; Hainea, D.; Morgan, G. *Jpn. J. Pharmacol.* **2000**, *83*, 241–245.
14. Louajri, A.; Harraga, S.; Godot, V.; Toubin, G.; Kantelip, J. P. *Biol. Pharm. Bull.* **2001**, *24*, 710–712.
15. Schulz, J.; Halama, P.; Hoerr, R. In *The Evolution, Ecology and Cultivation of Ginkgo biloba*; Harwood Academic: Amsterdam, 2000; Vol. 12.
16. Kennedy, D. O.; Scholey, A. B.; Wesnes, K. A. *Psychopharmacology* **2000**, *151*, 416–423.
17. Polich, J.; Gloria, R. *Hum. Psychopharmacol. Clin. Exp.* **2001**, *16*, 409–416.
18. DeFeudis, F. V.; Drieu, K. *Curr. Drug Targets* **2000**, *1*, 25–58.
19. Braquet, P.; Drieu, K.; Etienne, A. *Actual. Chim. Ther.* **1986**, *13*, 237–254.
20. Ishii, S.; Shimizu, T. *Prog. Lipid. Res.* **2000**, *39*, 41–82.
21. Kato, K.; Clark, G. D.; Bazan, N. G.; Zorumski, C. F. *Nature* **1994**, *367*, 175–179.
22. Bito, H.; Nakamura, M.; Honda, Z.; Izumi, T.; Iwatsubo, T.; Seyama, Y.; Ogura, A.; Kudo, Y.; Shimizu, T. *Neuron* **1992**, *9*, 285–294.
23. Klein, J.; Chatterjee, S. S.; Loffelholz, K. *Brain Res.* **1997**, *755*, 347–350.
24. Simonson, W. *Am. J. Health-Syst. Pharm.* **1998**, *55*, S11–S16.
25. Itil, T.; Martorano, D. *Psychopharmacol. Bull.* **1995**, *31*, 147–158.
26. Le Bars, P. L.; Katz, M. M.; Berman, N.; Itil, T. M.; Freedman, A. M.; Schatzberg, A. F. *J. Am. Med. Assoc.* **1997**, *278*, 1327–1332.
27. Warburton, D. M. *Br. J. Clin. Pharmacol.* **1993**, *36*, 137.
28. Buccafusco, J. J.; Terry, A. V. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 438–446.
29. Green, R. C. *Clin. Neurol.* **1995**, *3*, 1–83.
30. Stoppe, G.; Sandholzer, H.; Staedt, J.; Winter, S.; Kiefer, J.; Ruther, E. *Pharmacopsychiatry* **1996**, *29*, 150–155.
31. *The Complete German Commission E Monographs Therapeutic Guide to Herbal American Botanical Council*; Blumenthal, M., Busse, W. R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C., Rister, R. S., Eds.; American Botanical Council: Austin, Texas, 1998.
32. http://wwwbeaufour-ipsencom.
33. Rigney, U.; Kimber, S.; Hindmarch, I. *Phytother. Res.* **1999**, *13*, 408–415.

34. Huguet, F.; Drieu, K.; Piriou, A. *J. Pharm. Pharmacol.* **1994**, *46*, 316–318.
35. Huguet, F.; Tarrade, T. *J. Pharm. Pharmacol.* **1992**, *44*, 24–27.
36. Watanabe, C. M. H.; Wolffram, S.; Ader, P.; Rimbach, G.; Packer, L.; Maguire, J. J.; Schultz, P. G.; Gohil, K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6577–6580.
37. Weinges, K.; Hepp, M.; Jaggy, H. *Liebigs Ann. Chem.* **1987**, 521–526.
38. Nakanishi, K.; Habaguchi, K. *J. Am. Chem. Soc.* **1971**, *93*, 3546–3547.
39. Schwarz, M.; Arigoni, D. In *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Eds.; Elsevier: Oxford, 1999, pp 367–400.
40. Rohmer, M. In *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Eds.; Elsevier: Oxford, 1999, pp 45–67.
41. Schepmann, H. G.; Pang, J.; Matsuda, S. P. T. *Arch. Biochem. Biophys.* **2001**, *392*, 263–269.
42. Corey, E. J.; Su, W. G. *J. Am. Chem. Soc.* **1987**, *109*, 7534–7536.
43. Corey, E. J.; Gosh, A. K. *Tetrahedron Lett.* **1988**, *29*, 3205–3206.
44. Corey, E. J.; Kang, M. C.; Desai, M. C.; Ghosh, A. K.; Houpis, I. N. *J. Am. Chem. Soc.* **1988**, *110*, 649–651.
45. Crimmins, M. T.; Pace, J. M.; Nantermet, P. G.; Kim-Meade, A. S.; Thomas, J. B.; Watterson, S. H.; Wagman, A. S. *J. Am. Chem. Soc.* **2000**, *122*, 8453–8463.
46. Stromgaard, K.; Nakanishi, K. *Agnew. Chem. Int. Ed.* **2004**, *43*, 1640–1658.
47. Smith, J. V.; Luo, Y. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 465–472.
48. Woods, M. C.; Miura, I.; Nakadaira, Y.; Terahara, A.; Maruyama, M.; Nakanishi, K. *Tetrahedron Lett.* **1967**, *4*, 321–326.
49. Nakadaira, Y.; Hirota, Y.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1969**, 1469.
50. van Beek, T. A.; Lelyveld, G. P. *Phytochem. Anal.* **1993**, *4*, 261–268.
51. Lichtblau, D.; Berger, J. M.; Nakanishi, K. *J. Nat. Prod.* **2002**, *10*, 1501–1504.
52. Nakanishi, K., Jaracz, S., Malik, S., Ishii, H., Dzyuba, S. V.; PCT Int. Appl. WO 2005046829, **2005**, pp 61.
53. Tanaka, K.; Kester, K. D.; Berova, N.; Nakanishi, K. *Tetrahedron Lett.* **2005**, *46*, 531–534.
54. Tanaka, K.; Kester, K. D.; Berova, N.; Nakanishi, K. *Bull. Chem. Soc. Jpn.*, in press..
55. Ivic, L.; Sands, T. T. J.; Fishkin, N.; Nakanishi, K.; Kriegstein, A. R.; Stromgaard, K. *J. Biol. Chem.* **2003**, *278*, 49279–49285.
56. Jaracz, S.; Nakanishi, K.; Jensen, A. A.; Stromgaard, K. *Che, Eur. J.* **2004**, *10*, 1507–1518.
57. Maclennan, K. M.; Smith, P. F.; Darlington, C. L. *Prog. Neurobiol.* **1996**, *50*, 585–596.
58. Smith, P. F.; Maclennan, K. M. *Curr Opinion Anti-inflamm Immunomodul Invest Drugs* **1999**, *1*, 205–218.
59. Kondratskaya, E. L.; Pankratov, Y. V.; Lalo, U. V.; Chatterjee, S. S.; Kristal, O. A. *Neurochemistry International* **2004**, *44*, 171–177.
60. Kato, K. *Adv. Exp. Med. Biol.* **1999**, *469*, 221–227.
61. Kornecki, E.; Wieraszko, A.; Chan, J. C.; Ehrlich, Y. H. *J. Lipid Mediators Cell Signalling* **1996**, *14*, 115–126.
62. Bazan, N. G.; Allan, G. *J. Lipid Mediators Cell Signalling* **1996**, *14*, 321–330.
63. Dell' Albani, P.; Condorelli, D. F.; Mudo, G.; Amico, C.; Bindoni, M.; Belluardo, N. *Neurochem. Int.* **1993**, *22*, 561–574.
64. Doucet, J. P.; Bazan, N. G. *Mol. Neurobiol.* **1992**, *6*, 407–424.
65. Kobayashi, K.; Ishii, S.; Kume, K.; Takahashi, T.; Shimizu, T.; Manabe, T. *Eur. J. Neurosci.* **1999**, *11*, 1313–1316.
66. Chen, C.; Magee, J. C.; Marcheselli, V.; Hardy, M.; Bazan, N. G. *J. Neurophysiol.* **2001**, *85*, 384–390.
67. Krane, S.; Kim, S. R.; Abrell, L. M.; Nakanishi, K. *Helv. Chim. Acta* **2003**, *86*, 3776–3786.
68. Vogensen, S. B.; Stromgaard, K.; Shindou, H.; Jaracz, S.; Suehiro, M.; Ishii, S.; Shimizu, T.; Nakanishi, K. *J. Med. Chem.* **2003**, *46*, 601–608.
69. Stromgaard, K.; Saito, D. R.; Shindou, H.; Ishii, S.; Shimizu, T.; Nakanishi, K. *J. Med. Chem.* **2002**, *45*, 4038–4046.
70. Jaracz, S.; Stromgaard, K.; Nakanishi, K. *J. Org. Chem.* **2002**, *67*, 4623–4626.