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Reviews

Ginkgo biloba and Ginkgotoxin

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Products prepared from *Ginkgo biloba* are top-selling phytopharmaceuticals especially in Europe and major botanical dietary supplements in the United States. In European medicine, *G. biloba* medications are used to improve memory, to treat neuronal disorders such as tinnitus or intermittent claudication, and to improve brain metabolism and peripheral blood flow. The whole array of indications is reflected by a number of defined natural product constituents in *G. biloba*. The most well-known ones are flavonoids and terpene lactones, but they also include allergenic and toxic compounds such as ginkgotoxin (**1**). Consequently, there are reports attributing beneficial as well as adverse effects to *G. biloba* products. The present paper summarizes recent experiences with *G. biloba* and its derived products and explains why their restricted use is recommended.

Introduction

Fossil plants of the family Ginkgoaceae are well-known to paleobotanists. Petrified species of this family were alive 300 million years ago (Permian period) and attained their greatest prominence 200 million years before the present times (Jurassic period). During the Tertiary period (60 million years ago) all except two of the 19 genera with nearly 60 species became extinct. Today naturally occurring trees disappeared from all but one continent, on which only one single species, *Ginkgo biloba* L., survived.[1] Within the division of Spermatophyta, *G. biloba* belongs to the subdivision Coniferophytina, consisting of the classes Pinopsida and Ginkgoopsida, with the latter comprising only one order, the Ginkgoales, having only one family, the Ginkgoaceae, with only one genus, *Ginkgo,* and only one species, *G. biloba*.

The taxonomic position of *G. biloba* in the plant kingdom shows that it is a seed- and not a fruit-producing plant, a feature that is observed only in the subdivision of Magnoliophytina (Angiospermae). It is not in compliance with generally accepted botanical nomenclature to name the seeds of *G. biloba* "fruits", "nuts",[2,3] or even "pods",[4] as is often the case.

The Japanese botanist Hirase[3,5] was the first to describe the unique fertilization in which a multiciliated spermatozoid fuses with the egg cell, a process that *G. biloba* shares with the *Cycads*. The fertilization process leads to the formation of a seed with a sarcotesta and a sclerotesta housing the edible albumen (or endosperm) and the embryo. The *G. biloba* plant represents the highest developed taxon that still uses spermatozoids, which during evolution were abandoned when Pinopsida and Magnoliophytina appeared on the scene. The ancient morphological character of *G. biloba* also comes to light in the dichotomously branching veins in the leaf vascular tissue.

G. biloba has been thoroughly investigated for its constituents, and a whole array of compounds has been described.[6] These are the ginkgolides, bilobalide, ginkgolic acids, flavonoids (notably biflavonoids), triterpenes, carotenoids, polyprenols, essential oils, aromatic acids (*p*-hydroxybenzoic acid, protocatachuic acid, vanillic acid), ascorbic acid, D-glucaric acid, quinic acid, shikimic acid, alkyl coumarins, lipids, long-chain hydrocarbons, carbohydrates, glycerol derivatives, 1-hydroxypyrenes, zeatin, 6-hydroxykynuric acid, pentadiene-1,5-diphenol, tannins, and the highly toxic compound ginkgotoxin (**1**). The latter among all these compounds appears to be that exerting the greatest physiological activity in mammals, including humans.

Products from *Ginkgo biloba.* The *G. biloba* tree has long been held sacred for its therapeutic value. In mainland China and Japan preparations incorporating this species are used in the treatment of cough, bronchial asthma, irritable bladder, and even alcohol abuse[7] as well as for numerous additional indications in Japanese medicine.[8] Medications incorporating *G. biloba* leaves are used in European medicine in the treatment of insufficient blood flow, cerebral insufficiency, memory deficits, disturbances in concentration, depression, dizziness, tinnitus, vertigo, headache, intellectual deficit, and intermittent claudication.[2,9] Clinical trials on the efficacy of *G. biloba* medications are numerous and controversial.[10−12] The *Ginkgo* leaf active extract, EGb 761, was assessed in randomized, double-blind, placebo-controlled clinical trials for its influence on elderly patients suffering from[12] or developing[11] Alzheimer's disease. In the latter study, efficacy was not evident, whereas in the former investigation, seven individual studies were evaluated in a meta-analysis, resulting in the conclusion that a beneficial effect of EGb 761 is observed. It is disturbing, however, that the efficacy of EGb 761 in the individual studies performed varied considerably and that a beneficial effect was only observed in patients taking a 240 mg EGb 761 daily dose. No conclusion could be drawn for those on a 120 mg daily dose of EGb 761.[12] Thus, a dose-dependent influence on the health condition of patients was not demonstrated although the extract EGb 761 was standardized for its possible active ingredients, namely, terpene lactones (5−7%) and flavonoids (22−27%).

Among the natural products present in the *G. biloba* tree and its derived products, the ginkgolides are structurally unique in that they represent diterpene trilactones.[13−15] They co-occur with bilobalide, a sesquiterpene lactone.[16] These physiologically active terpenoid compounds are found only in this particular plant. Experiments on animal models showed that ginkgolides, notably ginkgolide B, inhibit platelet-activating factor and thus are able to influence the rheological properties of blood. Reduction of cerebral edema and improvement of brain blood flow after experimental stroke were attributed to the ginkgolides, especially ginkgolide B

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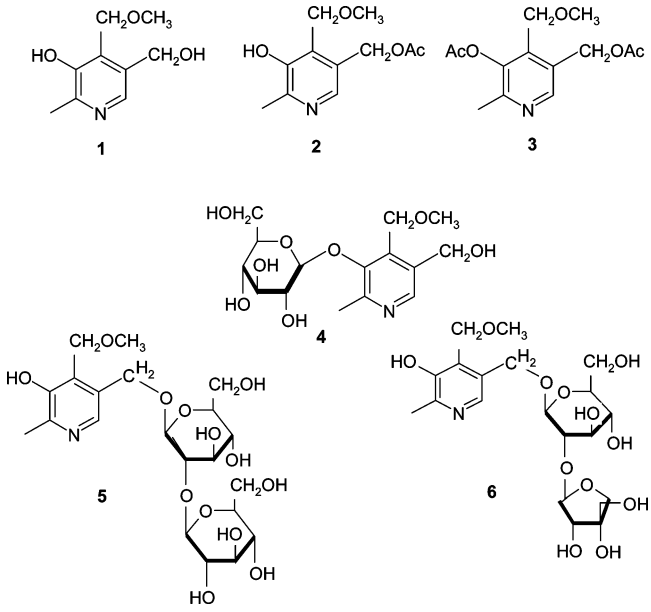

Figure 1. Structures of ginkgotoxin (4′-*O*-methylpyridoxine) (**1**), 5′-acetoxyginkgotoxin (**2**), 3,5′-diacetoxyginkgotoxin (**3**), ginkgotoxin 3-*O*-*β*-D-glucopyranoside (**4**), ginkgotoxin 5′-*O*-*β*-D-glucopyranosyl(1→2)*β*-D-glucopyranoside ("julibrin I") (**5**), and ginkgotoxin 5′-*O*-*β*-D-apiofuranosyl(1→2)-*β*-D-glucopyranoside ("julibrin II") (**6**). These compounds are constituents of *Ginkgo biloba* L. (**1**), *Albizzia tanganyicensis* Bak.f. (**1**, **2**), *Albizzia julibrissin* Durazz. (**4**−**6**), and *Albizzia lucida* Benth. (**3**, **4**).

(BN52021),[17] while bilobalide inhibits the breakdown of membrane phospholipids, a process that occurs during neurodegeneration.[10,18] However, the concentrations employed in these studies[18,19] exceed those used in clinical trials and therapeutic situations[20] by 2 to 3 orders of magnitude on a body-weight basis. Similar observations have also been noted by other authors.[10] A neuroprotective effect of the extract EGb 761 via stabilization of mitochondrial membranes and respiratory chain complexes has also been discussed.[21]

As is always the case, phytopharmaceutical preparations and botanical dietary supplements contain a whole array of natural products. Ginkgolic acids are ingredients of *G. biloba* medications, which in the German and the European Pharmacopoeas are limited to 5 ppm of the dried extract (EGb 761) because of their allergenic potential. Teas prepared from dried *G. biloba* leaves are also available on the market, and while the intake of such products is usually limited through the recommended daily dosage of a standardized medication, this limitation does not apply to *G. biloba* tea, in which the amount of ginkgolic acids in one cup may exceed the recommended upper limit by 80 times.[22] It is very likely that the tea contains also ginkgotoxin (**1**), which in the investigation cited[22] remained undetermined.

Ginkgotoxin (1): Structure and Biosynthesis. The structure of ginkgotoxin (**1**) (Figure 1), elucidated after activity-guided isolation using guinea pigs as a test system, was shown to be 4′-methoxypyridoxol,[23] which may occur also as an incompletely identified glycoside in *G. biloba* seeds.[24] Ginkgotoxin (**1**) and its naturally occurring derivatives have also been detected in *Albizzia tanganyicensis* Bak.f. (**1** and **2**),[25] *Albizzia lucida* Benth. (**3** and **4**),[26] and *Albizzia julibrissin* Durazz. (**4**, **5**, and **6**),[27] plants of the family Fabaceae (Figure 1).

The structure of ginkgotoxin (**1**) reflects its biosynthesis (Figure 2). The molecule is derived by 4′-*O*-methylation of pyridoxol (**11**) or its 5′-phosphate (**10**).[28] Experiments using a *G. biloba* cell suspension culture showed that D-[U-$^{13}C_6$]-glucose was incorporated into **1** after fragmentation of the labeled glucose into C_2 and C_3 units, resulting in a labeling pattern that is to be expected in vitamin B_6 biosynthesis.[29,30]

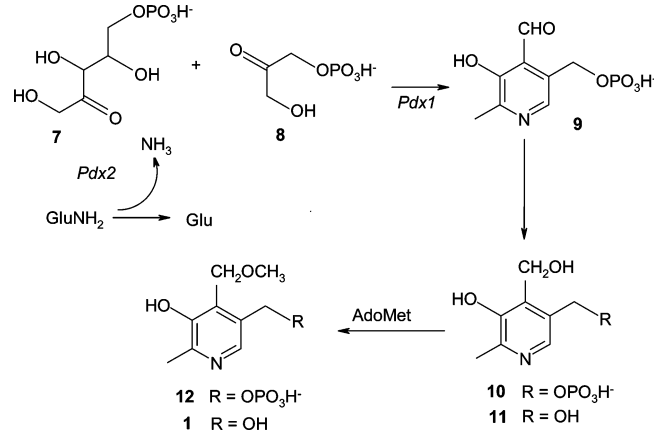

Figure 2. Biosynthesis of ginkgotoxin (**1**) or its 5′-phosphate (**12**) from ribulose 5-phosphate (**7**) (or ribose 5-phosphate) and dihydroxyacetone phosphate (**8**) (or glycerolaldehyde 3-phosphate) in the presence of the PLP synthase complex consisting of *Pdx1* and *Pdx2*. Since pyridoxal 5′-phosphate (**9**) is the first B_6 vitamer that is formed, a dehydrogenase reaction leading to pyridoxine (**11**) (pyridoxol) or its phosphate (**10**) has to be postulated. The 4′-*O*-methylating enzyme system accepts pyridoxine (**11**) as well as its 5′-phosphate (**10**) in the presence of *S*-adenosylmethionine (AdoMet).

The incorporation of label from Me-[$^{13}C_1$]-methionine into the 4′-*O*-methyl group of ginkgotoxin (**1**) was also observed.[28] These results, however, do not allow a distinction between the two similar but independent and essentially different vitamin B_6 biosynthetic pathways known today.[31−33] The two pathways were designated as the "deoxyxylulose-dependent" and "deoxyxylulose-independent" pathways.[31] Two genes, *pdxA* and *pdxJ*, are responsible for the biosynthesis of pyridoxol 5′-phosphate (**10**) in the *γ*-subdivision of proteobacteria including *Escherichia coli*.[34] Substrates for this process are deoxyxylulose 5-phosphate[30] and 4-phosphohydroxy-L-threonine,[35−37] with glutamate acting as the nitrogen donor.[38,39] Many attempts to incorporate isotopically labeled deoxyxylulose and its 5-phosphate into ginkgotoxin (**1**) in *G. biloba* cell suspension cultures and in enzyme preparations from *G. biloba* seeds have met with no success.[40,41] This contrasts with the deoxyxylulose-independent pathway, which is present in all archaea, fungi, plants, and most bacteria. In these organisms the vitamin B_6 biosynthesis is catalyzed by the gene products of *pdx1* and *pdx2* (Figure 2).[31−33]

Substrates for the biosynthesis are ribulose 5-phosphate (**7**) and dihydroxyacetone phosphate (**8**) (Figure 2). In this case, glutamine functions as the nitrogen donor, and the first detectable product is pyridoxal 5′-phosphate (**9**) rather than pyridoxol 5′-phosphate (**10**). The deoxyxylulose-independent process was shown to occur in *Bacillus subtilis* (Ehrenberg) Cohn,[42] *Arabidopsis thaliana* (L.) Heynh.,[43−45] *Plasmodium falciparum* Welch,[46] *Phaseolus vulgaris* (L.),[47] and *Nicotiana tabacum* (L.).[48]

During the biosynthesis the gene product *Pdx1* forms a dodecamer to which 12 *Pdx2* subunits attach. In solution *Pdx1* exists as a hexamer, being in equilibrium with its dodecameric form.[31] The *pdx1* gene is one of the most highly conserved genes. We have cloned this gene from *G. biloba*. While the *pdx1* gene exists in *A. thaliana* in three forms, named *Atpdx1.1*, *Atpdx1.2*, and *Atpdx1.3*, only one type of gene, termed *Gbpdx1*, seems to be present in *G. biloba.*[49] The *GbPdx1* gene product interacts with itself and with members of the *AtPdx1.1* and *AtPdx2* families. Moreover, the gene complements the *A. thaliana Atpdx1.3*-mutant (rsr-4-1) and reverses its vitamin B_6 deficiency at least partially.[45] This shows clearly that not only is the DNA sequence of the *Gbpdx1* gene highly conserved but also the functionality of its gene product *GbPdx1* in the dodecameric protein assembly system. Physiological experiments also show that the highest concentration of ginkgotoxin (**1**)

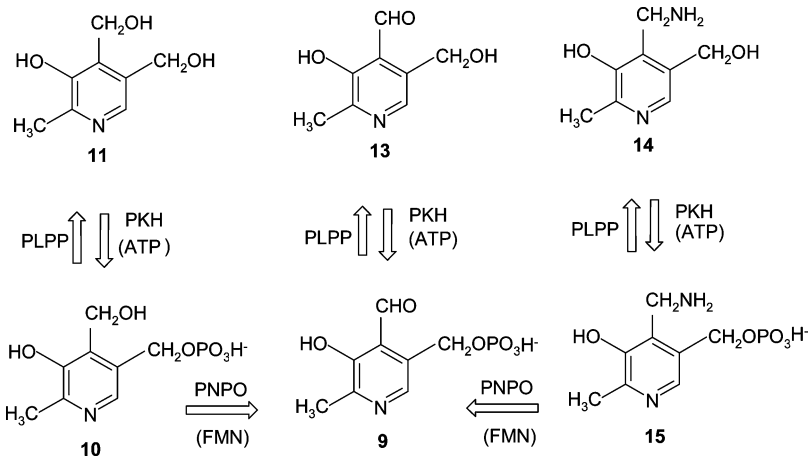

Figure 3. Human pyridoxalkinase (PKH), pyridoxal/pyridoxine/pyridoxamine 5′-phosphate phosphatase (PLPP), and pyridoxine/pyridoxamine 5′-phosphate oxidase (PNPO) are the enzymes interconverting the B_6 vitamers pyridoxine (**11**), pyridoxal (**13**), and pyridoxamine (**14**) and their respective phosphates (**10**, **9**, **15**) in the salvage pathway.

is observed in the seeds correlating with the highest expression of *Gbpdx1* when compared to leaf or trunk tissue of *G. biloba.*[49]

In conclusion, the evidence shows that ginkgotoxin (**1**) is structurally and biosynthetically a derivative of pyridoxol (**11**) formed by the deoxyxylulose-independent pathway.

Toxicology of Ginkgotoxin (1). The seed of *G. biloba* is called "gin-nan" in Japan, and although it is known to be a medication and a food, it is also the cause of "gin-an sitotoxism", a food poisoning that has been reported ca. 70 times with 27% lethality. These intoxications occurred between 1930 and 1996 with a rather high incidence during and after World War II when food was limited.

Children at the age of 1 to 3 years were affected in 58% of all reported cases of intoxication. At sublethal doses, symptoms of poisoning are eleptiformic seizures, unconsciousness, and paralysis of the legs.[50,51] A dosage of 11 mg/kg of the isolated ginkgotoxin (**1**) triggered seizures in guinea pigs. Administration of 30 to 50 mg/kg ip was followed by atrioventricular block or ventricular fibrillation and death of the animals.[23] An LD_{50} of ca. 30 mg/kg ip was determined for a rabbit. A higher dose of 400 to 600 mg/kg ip was needed to elicit convulsions in rats.[51]

The concentration of ginkgotoxin (**1**) in the albumen of *G. biloba* seeds increases during the vegetation period and reaches its maximum at the beginning of August with 85 μg of ginkgotoxin (**1**) per seed. Subsequently the ginkgotoxin (**1**) content declines rapidly.[52] A significantly higher concentration for seed ginkgotoxin (**1**) was reported by Scott et al.[24] and highly variable concentrations by Hori et al.[53] Ingestion of raw seeds seems to be most dangerous. Canned and boiled albumens contained only 1% of the ginkgotoxin (**1**) present in the raw seed. This may be attributed to the water solubility of ginkgotoxin (**1**).[52] Roasted seeds are also a dish in Japan and do contain ginkgotoxin (**1**).[54] One should assume that roasted seeds contain ginkgotoxin (**1**) at a concentration close to the amount present in raw seeds since this compound is rather stable. In Asian countries it is traditional to not eat too many *G. biloba* seeds during a single meal.[8]

The ginkgotoxin (**1**) levels present in blood serum of patients suffering seizures, tonic/clonic convulsions, vomiting, and impaired consciousness after ingestion of seeds have been reported.[53,54] Some examples are given: Thus, in four cases, male or female children at the age of one to two years had consumed 15 to 60 seeds. Convulsions were observed between 2 to 9 h after ingestion of seeds, resulting in serum concentrations from 0.24 to 1.28 μg/mL serum 3 to 8 h after eating the seeds. Yagi et al.[55] reported a concentration as low as 0.09 μg/mL serum level of **1** in a 21-months-old patient 8.5 h after ingestion of 50 *G. biloba* albumens. In another reported case, a 38-year-old woman had a serum level of 0.24 μg/mL 5 h after intake of 60 seeds. The amount of ginkgotoxin (**1**) declined to ca. 0.08 μg/mL within 35 h after ingestion.[53]

The presence of ginkgotoxin (**1**) and its derivatives in *Albizzia* species (Figure 1) is the cause of poisoning of livestock (cattle and sheep), which constitutes one of the most persistent and important agricultural problems in South Africa.[25] Basson et al.[56] reported on eight cattle in a herd of 160 that died and about 50 showed convulsions, the most characteristic symptoms of poisoning. The condition has been termed "albizziosis". The poisoning occurs in late winter and early spring when the pods are blown from the trees by strong winds. Young pods are most toxic, and as little as 0.57 to 1.14 kg of *Albizzia versicolor* Welw. ex Oliver pods proved fatal to cattle with body weights of ca. 230 kg.[57,58]

Steyn et al.[25] isolated 980 mg of ginkgotoxin (**1**) and 285 mg of 5′-acetyl 4′methoxypyridoxol (**2**) from 26 kg of *A. tanganyicensis* pods.

Molecular Basis of Ginkgotoxin Intoxication. Intoxications by ginkgotoxin (**1**) or *G. biloba* food can be counteracted by vitamin B_6.[23,34] A 30 mg dose (corresponding to 2 mg per kg body weight) of pyridoxal 5′-phosphate (**9**) administered to a child led to the recovery of the patient.[54] Survivors appear to suffer no serious post-seizure complications. Similarly, symptoms of poisoning in sheep that fed on *Albizzia* seedpods can also be cured in this way.[58] This indicates that ginkgotoxin (**1**) due to its structural similarity to vitamin B_6 interferes with metabolic steps related to the vitamin's function or biosynthesis.

Interestingly, a family with neonatal seizures has been described carrying a homozygous mutation of the gene encoding pyridoxol/pyridoxamine 5′-phosphate oxidase (PNPO) (Figure 3), suggesting that ginkgotoxin may cause seizures by interfering with the functioning of PNPO or the supply of vitamin B_6.[59] It is known that seizures can be triggered by vitamin B_6 deficiency.[60]

The PNPO gene is involved in the so-called salvage pathway (Figure 3) of vitamin B_6 biosynthesis in which B_6 vitamers (pyridoxol, **11**; pyridoxal, **13**; pyridoxamine, **14**; and their phosphates **10**, **9**, **15**) are interconverted. Although six metabolites participate in this metabolic sequence, only three enzymes are involved. These are pyridoxal/pyridoxol/pyridoxamine kinase (PKH) (EC 2.7.1.35), a flavine mononucleotide-dependent pyridoxol/pyridoxamine 5′-phosphate oxidase (PNPO) (EC 1.4.3.5), and pyridoxal/pyridoxol/pyridoxamine 5′-phosphate phosphatase (PLPP) (EC 3.1.3.3) (Figure 3).

The enzyme pyridoxol/pyridoxamine 5′-phosphate oxidase (PNPO) was investigated.[61] After overexpression of the human PNPO gene in *E. coli* the enzyme was purified to homogeneity by conventional affinity chromatography and characterized. The in vitro performance

of the enzyme, however, turned out not to be affected by ginkgotoxin (**1**) or its 5′-phosphate (**12**). The same observation was made for the overexpressed human pyridoxal/pyridoxol/pyridoxamine 5′-phosphate phosphatase (PLPP) (Figure 3).[61] The picture changed, however, after heterologous overexpression of the human kinase (PKH) gene in *E. coli* and purification again by conventional affinity chromatography of the kinase enzyme.[62] Characterization of the enzyme showed that the kinase phosphorylated not only the B_6 vitamers but also ginkgotoxin (**1**), and with a significantly lower K_m value (5.0×10^{-6} M) when compared to the enzyme's natural substrates, pyridoxal (**13**) (5.9×10^{-5} M), pyridoxol (**11**) (9.9×10^{-6} M), and pyridoxamine (**14**) (1.3×10^{-4} M). Co-incubation of both its natural substrate pyridoxal (**13**) and its nonnatural substrate ginkgotoxin (**1**) with the kinase revealed that phosphorylation of pyridoxal was almost nil as long as non-phosphorylated ginkgotoxin (**1**) remained in the incubation mixture, indicating a competitive inhibition of pyridoxal (**13**) phosphorylation by ginkgotoxin (**1**) due to its lower K_m value.

Computer modeling[62] of the active site of the kinase based on the known X-ray chrystallographic data for the sheep pyridoxal kinase[63] and the known amino acid sequence of the human pyridoxal kinase (PKH)[64] showed that the human kinase has a lipophilic pocket at the active site and that the lipophilic 4′-*O*-methyl group of ginkgotoxin (**1**) reaches into this pocket. This may explain why the K_m value for ginkgotoxin is significantly lower than those for the B_6 vitamers. This and the higher lipophilicity of ginkgotoxin (**1**) (log P −0.299) when compared to pyridoxal (**13**) (log P −1.182),[62] and hence the possibly enhanced capability of **1** to penetrate the blood brain barrier, has consequences for the impact of **1** on the human neuronal condition (see below).

Another observation is that before the onset and during eleptiformic seizures a relatively high level of glutamate and a low amount of γ-aminobutyrate is observed in the brain.[65−68] The imbalance between the two neurotransmitters is known to trigger epileptic convulsions. This led to the assumption that ginkgotoxin may inhibit the glutamate decarboxylase (EC 4.1.1.15).[66−70]

Indeed, glutamic acid decarboxylation is a delicate and crucial reaction, because it converts the most important neurotransmitter at excitatory synapses (glutamate) to the most important neurotransmitter at inhibitory synapses (γ-aminobutyrate) in an irreversible reaction.[71] In humans, two genes are responsible for this decarboxylation. They are annotated *gad65* and *gad67*, are located at chromosomal positions 10p11.23 and 2q31, respectively, and encode glutamate decarboxylases of different molecular weight.[72]

These observations led us to test the performance of overexpressed human *Gad65* and *Gad67* in the presence of ginkgotoxin (**1**) and its phosphate ester (**12**).[72] Overexpression was carried out in *E. coli* as a glutathione-*S*-transferase (GST) fusion protein, and the purified decarboxylases were characterized before and after proteolytic removal of the GST domain. Alternatively, the glutamate decarboxylases were overexpressed as histidine-tagged proteins in yeast. Kinetic properties of the proteins from different sources and different purification procedures were in reasonable agreement and matched published data obtained from other organisms.[73]

Most importantly, *Gad65* was obtained as the apoenzyme, while part of *Gad67* was present as the holoenzyme. This feature of both *Gad* enzymes reflects the regulation of glutamate decarboxylase activity by the supply of the cofactor pyridoxal 5′-phosphate. Indeed, the *Gad* enzymes are unique among pyridoxal 5′-phosphate-dependent enzymes in that they are regulated by the supply of their cofactor.[75−77]

The *Gad65* activity was reduced to 50% in vitro in the presence of ginkgotoxin 5′-phosphate (**12**) at a concentration of 2.7 mM.[73] Since a concentration as low as 1.28 μg/mL in the serum corresponding to 7 μM was found to cause seizures,[53] a 2.7 mM concentration for 50% in vitro inhibition (IC_{50}) of the human *Gad65* enzyme activity is considered as nonphysiologically high.[73] We conclude that in humans ginkgotoxin 5′-phosphate (**12**) is unlikely to trigger the symptoms of intoxication by direct inhibition of *Gad* enzymes.[53,67−70] The other human decarboxylase, *Gad67*, was even less affected by ginkgotoxin 5′-phosphate (**12**).[73] The activity of both *Gad* enzymes remained also unaffected by ginkgotoxin (**1**) itself.[73]

Several lines of evidence suggest that the two forms of *Gad65* and *Gad67* play different roles in the decarboxylation of glutamic acid in γ-aminobutyrate-containing neurons in the brain.[74−76] While *Gad65* is targeted to membranes in the nerve endings, *Gad67* is more widely distributed in cells. Both forms can synthesize γ-aminobutyrate, but *Gad67* might preferentially synthesize cytoplasmic material while *Gad65* is likely to decarboxylate glutamic acid for vesicle release of γ-aminobutyrate.[74−76] The interaction of *Gads* with its cofactor pyridoxal 5′-phosphate (**9**) is a process that plays a major role in the short term regulation of *Gad* activity. *Gad65* is more responsive than *Gad67* to the presence of the cofactor.

Our data show[61,62,73] that the primary event in ginkgotoxin (**1**) poisoning is not a direct effect on glutamate decarboxylase[53,67−70] but the inhibition of vitamin B_6 phosphorylation, depleting the brain of phosphorylated B_6 vitamers that may result in the down-regulation of glutamate decarboxylation. Kaufman et al.[74] have stated that modulation of holo-Gad65 levels at synapses may be responsible for the observed coupling of neuronal activity and *Gad* enzymatic activity. Thus, compound **1** exerts its influence on glutamate decarboxylation only indirectly. We are aware, however, that *Gad* activity is a highly sophisticated process that is regulated at different levels, such as transcription, regulation of mRNA, translation, protein stability, and enzymatic catalysis.[75] The direct inhibition of the *Gad* enzymes by ginkgotoxin (**1**) was an attractive model, which, however, is not supported by the published data.[62,73,75]

It is one of the strengths of molecular biology that experiments can be carried out on homogeneous purified human enzymes[61,62,73] as opposed to crude brain homogenates from experimental animals,[53,67−70] which make it difficult to distinguish between the two *Gad* enzymes and to avoid uncontrolled side effects.

Our observations on the impact of ginkgotoxin (**1**) on human brain metabolism are summarized in Figure 4. Ginkgotoxin (**1**) and B_6 vitamers are distributed by the bloodstream after passive intestinal absorption[59] and partly phosphorylated (path A). Before passage through the blood−brain barrier, phosphorylated metabolites are hydrolyzed (path B). Due to its higher lipophilicity, ginkgotoxin (**1**) is likely to be taken up preferentially through the blood−brain barrier and preferentially phosphorylated in the kinase reaction due to its relatively low K_m value with a simultaneously reduced posphorylation of B_6 vitamers.

This may result in a temporary depletion of pyridoxal 5′-phosphate (**9**) from the neuron, an impaired glutamate decarboxylation (path E), and seizures triggered as a consequence of an imbalance between the two neurotransmitters involved, as manifested by a relatively high glutamate and a low γ-aminobutyrate level.

The reaction of the kinase (PKH) is the first metabolic step that makes pyridoxal 5′-phosphate (**9**) available to the brain. The K_m value determined in vitro for the kinase and its non-natural substrate ginkgotoxin (**1**) (4.95 μM)[62] matches the ginkgotoxin plasma levels (1.31 to 6.99 μM) of those patients suffering from epileptic convulsions due to the consumption of *G. biloba* seeds.[53] Thus, in humans these results clearly implicate pyridoxal 5′-kinase (PKH) as a physiological target for ginkgotoxin (**1**) and provide a plausible explanation for the intoxication symptoms caused by this compound. However, it cannot be ruled out that hitherto unknown transport processes or any other vitamin B_6-dependent metabolic steps may also be affected by ginkgotoxin (**1**) or its 5′-phosphate (**12**). More than 100 vitamin B_6-catalyzed reactions are known.[77]

Occurrence of Ginkgotoxin (1) in *G. biloba* Products.

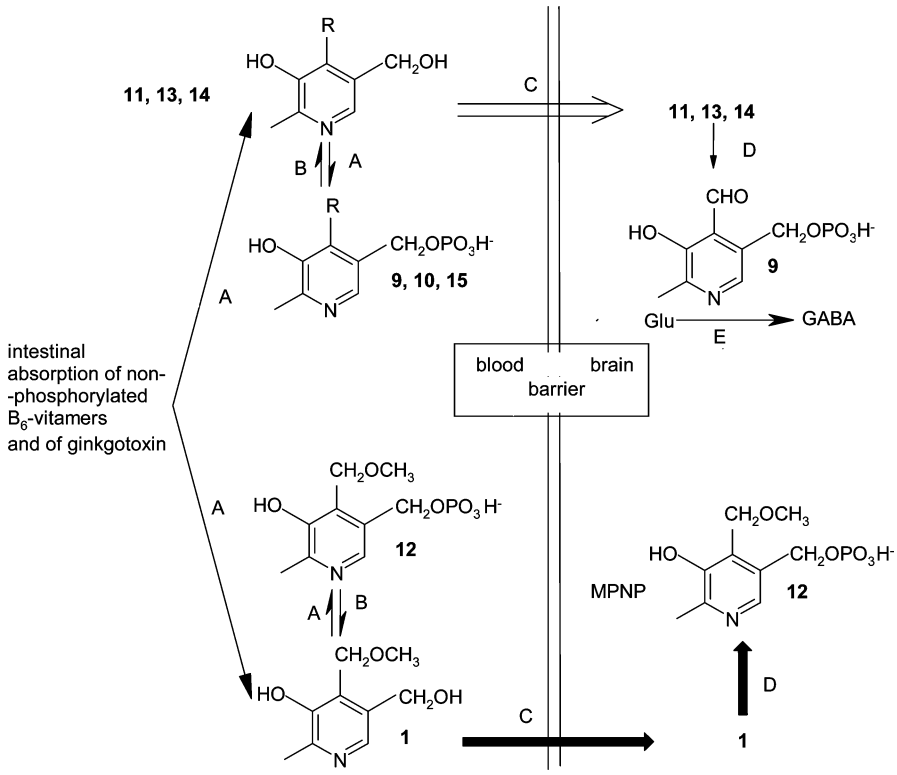

Figure 4. Suggested scheme summarizing metabolic reactions of ginkgotoxin (**1**), ginkgotoxin 5′-phosphate (**12**), and B_6 vitamers (pyridoxine, **11**; pyridoxamine, **14**; pyridoxal, **13**) and their respective 5′-phosphates (**10**, **15**, **9**) as catalyzed by human pyridoxal kinase (PKH), human pyridoxal 5′-phosphate phosphatase (PLPP), and human glutamate decarboxylases *GAD65* and/or *GAD67* in mammals including humans.[60] Reproduced with permission from Thieme, Stuttgart, Germany.

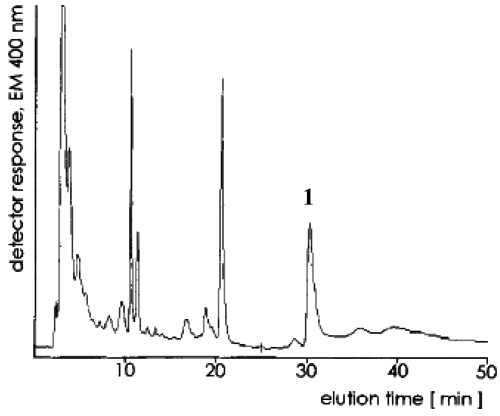

Figure 5. HPLC trace of a homeopathic *Ginkgo* preparation ("Ginkgo Loges Tropfen") (**1**; 4′-*O*-methylpyridoxine).[51] Reproduced with permission from Thieme, Stuttgart, Germany.

Ginkgotoxin (**1**) is a constituent not only of *G. biloba* seeds but also of *G. biloba* leaves. The highest concentration is observed with 5 μg per leaf, corresponding to ca. 7 μg/g fresh weight in leaves harvested at the beginning of August. Since *G. biloba* medications and botanical dietary supplemental products are derived from the leaves, they do also contain ginkgotoxin (**1**).[52] From 400 mL of the *G. biloba* medication Tebonin forte, containing ca. 3.25 mg of **1** (determined by HPLC, compare Figure 5), 2.9 mg was isolated preparatively by ion- exchange, paper, and thin-layer chromatography. The identity of the material was confirmed by ^{1}H and ^{13}C NMR spectroscopy and by comparison with a synthetic sample of **1**.

A HPLC analysis (Figure 5) revealed that different allopathic medications offered by various companies contain between 11.4 and 58.62 μg of ginkgotoxin in a recommended daily dose and 0.09 to 11.92 μg of **1** in homeopathic preparations. The daily amount of ginkgotoxin applied by injection of liquid medications ranged from 0.012 to 0.062 μg.[52]

The presence of ginkgotoxin (**1**) in *G. biloba* medications and other products raises the question if this could cause any undesirable health effects such as seizures. Assuming in a worst case scenario that all ginkgotoxin (**1**) (58.6 μg) taken up by one daily dose ends up in the blood serum, a concentration of **1** in human plasma of 53 to 80 nM calculated for 6 or 4 L of blood, respectively, would result. This is on the same order of magnitude as vitamin B_6 levels in blood plasma, which are reported to be 114 nM.[78]

At present it cannot be ruled out that *G. biloba* medications and other products may lower the threshold for seizures in epileptic patients. Strikingly, two cases of recurrence of well-controlled epilepsy after intake of *G. biloba* medications by two elderly patients have been reported.[79] After the immediate withdrawal of the plant remedy, in both cases no further epileptic convulsions were reported within the observation interval (8 months, first patient; 4 months, second patient).

In an attempt to put health care professionals on alert, Gregory[80] mentions seven cases listed by the U.S. Food and Drug Administration's Special Nutritionals Adverse Event Monitoring System (SN/AEMS) because of seizures related to *G. biloba*. The SN/AEMS system is currently under reconstruction.[81] Other anecdotal reports come from Internet discussion boards and electronic mailing lists.[82] Although different authors discuss the potential adverse effects of *G. biloba* products,[83−88] a causality could not be firmly established between these remedies and seizures because only a few cases have come to light.

Contrary to our own previous assumption[52] we are now convinced, however, that *G. biloba* medications and other products can have a detrimental effect on a person's health condition. This conclusion comes not only from the presence of **1** in medications and not only from the now known effect of **1** on the action of pyridoxal/pyridoxol/pxridoxamine kinase, a pivotal reac-

tion responsible for the supply of the brain with pyridoxal 5′-phosphate (**9**), but also from reports in which it was shown that *G. biloba* leaf extract exerts a proconvulsive effect on mammals including rabbits[69] and mice.[88]

In addition, some important observations on the interaction of *G. biloba* products with anticonvulsant and other medications have been published. In a case report, Kupiec et al.[88] described a patient who took both anticonvulsant medicine and simultaneously *G. biloba* products. The patient's blood serum levels of the anticonvulsants dropped below the therapeutic range and thus diminished the resulting therapeutic benefit. The patient died while swimming at a health club. The low level of anticonvulsants was attributed to the induction of cytochrome P450 enzymes by the *G. biloba* products.

In a similar line of reasoning Yin et al.[89] report on a series of experiments with 18 healthy male Chinese subjects who were simultaneously treated with omeprazol, a widely used proton pump inhibitor, and a *G. biloba* leaf extract specified according to guidelines set by the German Commission E responsible for phytopharmaceuticals. The results showed that the leaf extract induces omeprazol hydroxylation in a CYP2C19 genotype-dependent manner. This offers an explanation for the induction of seizures in predisposed patients with medically controlled epilepsy. Thus, it is possible that *G. biloba* products that are complex mixtures induce specific cytochrome P450 enzymes, which may accelerate the degradation of antiepileptic drugs. This seems very plausible in light of the fact that numerous natural products are present in *G. biloba* (see above) and its derived products. Bilobalide has been discussed as an inducer of metabolizing enzymes.[90]

The Bundesinstitut für Pharmazie and Medizinprodukte (Bfarm) in Bonn, the German institution corresponding to the U.S. Food and Drug Administration, FDA, ruled recently that pharmaceutical companies selling *G. biloba* medications have to amend the package insert advising patients suffering from seizures to consult their physician before taking high-dosage medications of this type. Physicians have to be informed upon request that an adverse effect of ginkgotoxin (**1**) is possible.

Conclusion

In summary, the foregoing discussion shows that in addition to the presumed, but not unequivocally proven, beneficial health effects of *G. biloba* products, these preparations also carry a clear potential for adverse effects, particularly in susceptible individuals. It is therefore important that the large number of *G. biloba* product users and their health care providers be made aware of these risks, in order to enable them to make informed decisions about the use of these preparations. In addition, it would be desirable for manufacturers/distributors of *G. biloba* preparations to assay their products not only for the levels of terpene lactones and flavonoids, as part of their standardization procedures, but also for the concentration of ginkgotoxin (**1**) as a measure of the safety of their product.

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