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Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 13 (2005) 5001–5012

Review

# Ginkgolides and bilobalide: Their physical, chromatographic and spectroscopic properties

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Received 8 April 2005; accepted 26 May 2005
Available online 29 June 2005

Dedicated to Professor Koji Nakanishi, who is unsurpassed in actively contributing to the isolation, purification, structure elucidation, spectroscopy, chemical conversion and pharmacology of "his" ginkgolides during the past 40 years.

**Abstract**—Ginkgolides A, B, C, J, K, L and M and bilobalide are rare terpene trilactones that have been isolated from leaves and root bark of the Chinese tree *Ginkgo biloba*. The structures of the highly oxidized ginkgolides were independently elucidated in the 1960s by the groups of Nakanishi and Sakabe. Later these compounds were found to be potent and selective antagonists of platelet activating factor, which fact triggered much new research. During the past 40 years, much physical, chromatographic and spectroscopic data have been published on these compounds in various, sometimes inaccessible, sources. The published melting points, solubility in different solvents, ionization constants, chromatographic behaviour, specific optical rotations, UV, IR, MS and NMR data, and X-ray studies are summarized and, where necessary, discussed. The literature until April 2005 has been reviewed.


## Contents



*Keywords*: Ginkgo terpene trilactones; *Ginkgo biloba*; Solubility; $[\alpha]_D$; UV; IR; MS; NMR; X-ray.
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doi:10.1016/j.bmc.2005.05.056

## 1. Introduction

*Ginkgo biloba* L. is the only remaining species of the once large order Ginkgoales. Its extraordinary botanical position and other peculiar properties have attracted, since long, the attention of scientists of different disciplines, for example, botanists, entomologists, natural products chemists and pharmacologists. In the 1960s, the groups of Nakanishi and co-workers[1–5] and Sakabe[6,7] independently isolated from, Ginkgo root bark and Ginkgo leaves, respectively, a so far unique class of plant constituents. They called them ginkgolides. These C20 terpenes consist of six five-membered rings, that is, a spiro[4,4]nonane carbocylic ring, three lactones and a tetrahydrofuran (**1**–**7**). Until now they are the only natural products possessing a *tert*-butyl group. Nakanishi has reviewed the structure elucidation of the ginkgolides elsewhere.[8–11] Originally ginkgolide A (**1**), B (**2**), C (**3**) and M (**4**) were identified.[8] In 1987, Weinges et al. identified ginkgolide J (**4**) as a minor constituent in the Ginkgo leaves.[12] Recently, Wang et al. reported on two new trace ginkgolides and called them ginkgolide K (**6**) and L (**7**).[13]

In the mid-1980s, it was discovered that ginkgolides were potent and selective antagonists of platelet activating factor (PAF).[14,15] PAF is a highly active mediator in the human body and has been implicated in various disease states. This discovery rapidly led to an enormous increase in the number of papers devoted to all aspects of ginkgolides (Fig. 1). So far four books have appeared on *Ginkgo biloba* and its chemical constituents.[16–19] As a result of the intense pharmacological and clinical research, phytopharmaceuticals based on partially purified *Ginkgo biloba* leaf extracts are now among the most sold drugs in France and Germany. Total worldwide sales of finished products were estimated at half a billion US dollars in 2000.[20] These extracts, containing 24% flavonol glycosides and 6% terpene trilactones, are primarily prescribed for problems associated with a poor central and peripheral blood circulation like mild forms of dementia and difficulties with walking, respectively.[21] A positive clinical trial for the treatment of Alzheimer's disease with a Ginkgo extract has been reported.[22] Although constituents from different chemical classes contribute to the overall pharmacological effect of Ginkgo extracts, ginkgolides are held responsible for a significant part of the beneficial effect. More recently, it was discovered that, apart from PAF-antagonism, they also exert antagonistic effects on glycine receptors[23–25] and an anxiolytic effect.[26] A closely related C15 terpene trilactone, which is the major terpene in Ginkgo leaves, is named bilobalide (**8**). Its structure was elucidated due to a concerted effort of Nakanishi and colleagues shortly after the discovery of ginkgolides A, B, C and M.[27] This terpene trilactone lacks PAF antagonistic activity but shows neuroprotective effects.[28,29]

According to a literature search with SciFinder Scholar, in total 1363 papers and patents appeared in the period 1965–2005 in which either ginkgolide A [15291-75-5] (**1**), ginkgolide B [15291-77-7] (**2**), ginkgolide C [15291-76-6] (**3**), ginkgolide J [107438-79-9] (**4**), ginkgolide K [153355-70-5] (**6**), ginkgolide L [148637-23-4] (**7**), ginkgolide M [15291-78-8] (**5**) or bilobalide [33570-04-6] (**8**) occurred (Fig. 1a). A minor part of these papers deals with their analysis or spectroscopic data (Fig. 1b). Somewhat surprisingly however, not a single review has yet appeared, which deals exclusively with all the physical and spectroscopic data of Ginkgo terpene trilactones. The sometimes conflicting data on their solubility in various solvents, melting points, ionization constants, TLC $R_f$ values, HPLC capacity factors, GC retention indices, specific optical rotations and IR, UV, MS, $^1$H NMR, $^{13}$C NMR and X-ray data have appeared over a period of 40 years in many different—sometimes inaccessible—sources. These data of all the eight presently known Ginkgo terpene trilactones are summarized below and commented on, if deemed necessary. If identical data have been published multiple times, only the firstly published data will be presented unless the newer data are better. Throughout the remaining part of this paper ginkgolides A, B, C, J, K, L and M will be abbreviated as G-A, G-B, G-C, G-J, G-K, G-L and G-M, respectively. Bilobalide will be abbreviated as BB.

## 2. Appearance and physical data

### 2.1. Taste and appearance

Ginkgolides in pure form occur in the form of white odourless crystals or powders. They have a bitter taste.[1]

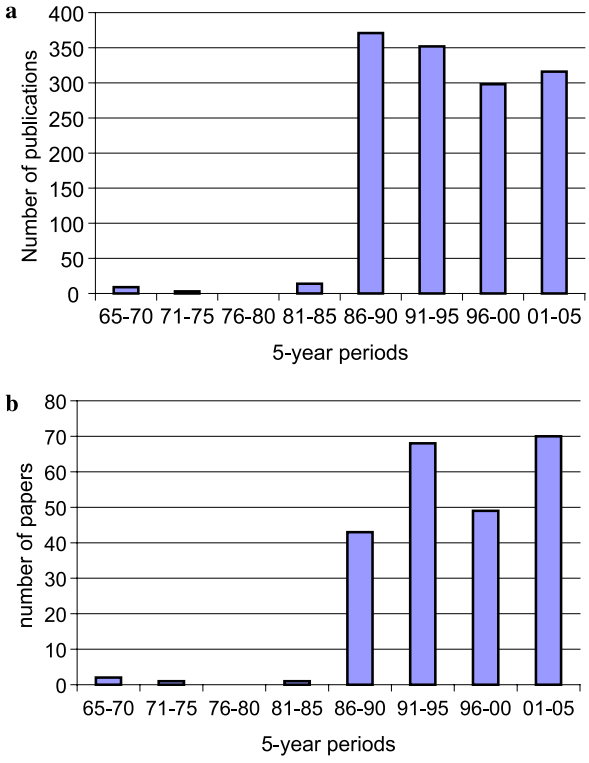
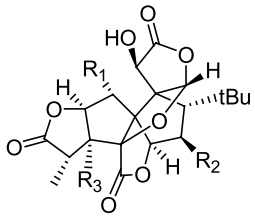
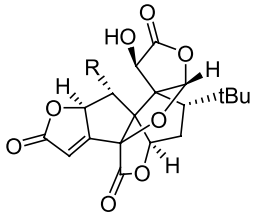



**Figure 1.** (a) Number of papers per 5-year period in which Ginkgo terpene trilactones occur. Based on a literature search with SciFinder Scholar. 65–70 means the period from 1965 to 1970. (b) Number of papers per 5-year period dealing with the analysis and spectroscopic properties of Ginkgo terpene trilactones. Based on a literature search with SciFinder Scholar. 65–70 means the period from 1965 to 1970.

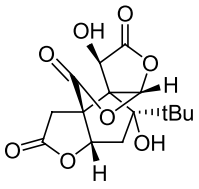
They are stable to light and moisture.[30] BB (**8**) is also reported to be bitter.[31]



1. Ginkgolide A: $R_1 = R_2 = H$, $R_3 = OH$
2. Ginkgolide B: $R_1 = R_3 = OH$, $R_2 = H$
3. Ginkgolide C: $R_1 = R_2 = R_3 = OH$
4. Ginkgolide J: $R_1 = H$, $R_2 = R_3 = OH$
5. Ginkgolide M: $R_1 = R_2 = OH$, $R_3 = H$



6. Ginkgolide K: R = OH
7. Ginkgolide L: R = H



8. Bilobalide

### 2.2. Melting points

Ginkgolides are relatively stable substances, which contain a large number of functional groups and, therefore, possess high melting points. Their melting points have been summarized in Table 1. There are significant differences in the reported melting points which must be due to the solvent used for crystallization, purity and experimental conditions. Melting points are thus of limited value for identification or purity assessment of ginkgolides. Terahara has stated that G-A (**1**), G-B (**2**) and G-C (**3**) can be sublimed at $10^{-3}$ mmHg at temperatures of 280–300 °C.[30]

**Table 1.** Reported melting points of ginkgolides and bilobalide

| Compound | Melting point (°C) | Reference |
|---|---|---|
| G-A, G-B, G-C | 330–350 (with decomposition) | 30 |
| G-A, G-B, G-C | ~300 | 6 |
| G-A, G-B, G-C, BB | >300 | 31 |
| G-A | 310; 332–335 | 32,33 |
| G-B | 295–297; 295–298 | 34,35 |
| G-B | 301 | 36 |
| G-C | 310; >300; 285 | 32,33,35 |
| G-J | 320 (decomposing from 295) | 12 |
| G-J | 290 | 32 |
| G-K, G-L | >300 | 13 |
| G-M | 320–324 (with decomposition) | 37 |
| BB | 300 | 34 |

### 2.3. Solubilities

Ginkgolides are soluble in acetone, ethanol, methanol, ethyl acetate, tetrahydrofuran, dioxane, acetic acid, trifluoroacetic acid, acetonitrile, pyridine and dimethyl sulfoxide. They are sparingly soluble in ether and water. They are insoluble in hexane, benzene, chloroform and carbon tetrachloride. Ginkgolides are extremely stable towards mineral acids, for example, evaporation to dryness of a concentrated nitric acid solution results in recovery of crystalline starting material, and dissolution of the ginkgolides in warm concentrated sulfuric acid followed by addition of water affords unchanged ginkgolides. Ginkgolides readily dissolve in dilute alkali and are recovered quantitatively on subsequent acidification.[38]

Mixtures of acetone–water or methanol–water have been frequently used to extract terpene trilactones from leaves indicating a good solubility in these mixed solvents.[39–50] They can also be extracted by supercritical carbon dioxide modified with 10% methanol at 335 atm and 45 °C.[51] A discussion of the extraction of terpene trilactones from leaves has been published.[52]

Specific solubility data on BB (**8**) are not available but its polarity and solubility appears to be similar to those of the ginkgolides. For instance, with the mixed solvents mentioned above it is well extracted. An exception is its instability above pH 7.[53,54]

### 2.4. Water/*n*-octanol partition coefficient (*P*)

A log *P* value of 1.72 was reported for G-B (**2**).[55]

### 2.5. Ionization constants

The following three $pK_a$ values were reported for G-B (**2**): $pK_{a1} = 7.14$, $pK_{a2} = 8.60$ and $pK_{a3} = 11.89$.[55] These are due to the presence of the three lactone rings. That one of the lactone rings is more difficult to open with base, had already been noted by Nakanishi.[8]

## 3. Chromatographic retention

### 3.1. TLC on silica gel

Terpene trilactones are poorly resolved on normal silica gel. BB (**8**) has the highest $R_f$ value, followed by G-A/G-B as a pair and G-J/G-C as a pair. The poor separation of G-A (**1**) and G-B (**2**) is caused by the strong hydrogen bonding between the 1- and 10-hydroxyl groups in G-B. Due to the hydrogen bonding, G-B is equal in polarity to G-A in normal phase chromatography in spite of the presence of an extra hydroxyl group. Exactly the same phenomenon occurs with G-J (**4**) (3 hydroxyls) and G-C (**3**) (4 hydroxyls), which possess $R_f$ values of

0.20 and 0.19, respectively, in toluene/acetone (7:3), a TLC solvent that was used by the group of Weinges et al.[12]

A complete separation of BB, G-A, G-B, G-J and G-C can be obtained if the silica gel plates are impregnated with sodium acetate prior to development. As solvent, pure methyl acetate was used.[56] Supposedly the sodium acetate disrupts the internal hydrogen bonding in G-B (**2**) and G-C (**3**), which results in a much better resolution of the five major Ginkgo terpene trilactones. The compounds now elute according to the type and number of their hydroxyl groups: BB (one 2°-OH and one 3°-OH, $R_f = 0.58$), G-A (one 2°-OH and one 3°-OH, $R_f = 0.41$), G-B (two 2°-OH and one 3°-OH, $R_f = 0.25$), G-J (two 2°-OH and one 3°-OH, $R_f = 0.12$) and finally G-C (three 2°-OH and one 3°-OH, $R_f = 0.06$).[56] The ginkgolides are probably more retained than BB because of the additional tetrahydrofuran ring present.

### 3.2. Reversed-phase HPLC

A thorough study on finding the optimal solvent for the separation of G-A, G-B, G-C and BB on C18 columns was carried out as far back as 1983. The results are summarized in Table 2. According to these studies the optimal solvent was methanol/tetrahydrofuran/water (1:3:15). At a flow rate of 1.5 mL/min this solvent gave a good separation of BB, G-A, G-B and G-C within 20 min.[57]

A further optimized solvent was published by Teng.[58] His separation took place on a 125 × 4 mm Merck RP C18 column at 30 °C and was completed within 9 min in combination with the solvent water/methanol/tetrahydrofuran (7:2:1) at 1.0 mL/min. The capacity factors ($k'$) for G-J (**4**), G-C (**3**), BB (**8**), G-A (**1**) and G-B (**2**) were 1.85, 2.62, 3.30, 4.29 and 6.05. The solvent tetrahydrofuran has a significant influence on the retention of BB (**8**) relative to the ginkgolides.

### 3.3. Gas chromatography

Ginkgolides and bilobalide are too involatile to be analysed as such by gas chromatography. However, after silylation they can be easily analysed by gas chromatography on a non-polar column. The retention indices of silylated BB (**8**) and G-A (**1**), G-B (**2**) and G-C (**3**) on a dimethylpolysiloxane phase at three different temperatures have been reported and are given in Table 3.[59]

**Table 3.** Retention indices of the trimethylsilyl ethers of ginkgolides A–C and bilobalide[59]

| Compound | 290 °C | 300 °C | 310 °C |
|---|---|---|---|
| BB | 2481.9 | 2504.2 | 2526.7 |
| G-A | 3130.7 | 3153.1 | 3175.6 |
| G-B | 3211.0 | 3235.0 | 3259.0 |
| G-C | 3241.9 | 3263.4 | 3285.0 |

## 4. Spectroscopic data

### 4.1. Specific optical rotation

In Table 4 the specific optical rotation of all ginkgolides and bilobalide at 589 nm are given.

### 4.2. Ultraviolet (UV) spectra

Due to the absence of carbon–carbon double bonds, ginkgolides A, B, C, J and M show only a weak maximum around 220 nm. Okabe et al. reported the following $\lambda_{max}$ (EtOH) values: G-A (**1**): 219 nm (log ε 2.72); G-B (**2**): 219 nm (log ε 2.37); G-C (**3**): 220 nm (log ε 2.30).[6] G-L (**7**): 217 nm (ε 10,500), 238 nm (sh, ε 6000).[38] Due to the low ε values, UV is unsuitable for detection purposes after HPLC and instead refractive index (RI), evaporative light scattering (ELSD) or MS detection should be used.

### 4.3. Optical rotatory dispersion (ORD) spectra

Ginkgolides show negative plain ORD curves exhibiting no Cotton effect in the range 250–700 nm.[8,38]

### 4.4. Infrared (IR) spectra

A detailed study of the IR spectra of G-A (**1**), G-B (**2**) and G-C (**3**) has been published by Broquet and Braquet.[61] They recorded standard spectra in nujol and Fourier Transform spectra in KBr. All the spectra were very complex. For a reliable assignment of the stretch vibrations of the hydroxyls, some spectra were recorded after deuteration with $CD_3OD$ as well. In Tables 5a–c the most characteristic signals are presented together with the assignment suggested by Broquet and Braquet. Non-characteristic bands such as the C–C stretch around 2900 $cm^{-1}$ have been omitted.

The stretching bands in the region between 1200 and 1050 $cm^{-1}$ were very difficult to assign because of the large number of C–O bonds in the ginkgolides. One of these bands could possibly correspond with the C–O–C moiety of the tetrahydrofuran ring (ring D), which undergoes conformational changes according to the degree of substitution. The precise stereochemistry at C-1

**Table 2.** HPLC retention times in minutes of ginkgolides A–C, and bilobalide on LiChrosorb RP-18 10 μm, 25 × 0.4 cm

| Solvent | Flow (mL/min) | BB | G-C | G-A | G-B |
|---|---|---|---|---|---|
| Methanol/water (3:7) | 1 | 8.8 | 10.6 | 21.4 | 24.2 |
| Acetonitrile/water (1:4) | 1 | 10.0 | 13.6 | 27.0 | 27.0 |
| Tetrahydrofuran/water (1:4) | 1 | 24.8 | 17.2 | 23.2 | 37.4 |
| Methanol/tetrahydrofuran/water (1:3:15) | 1.5 | 13.6 | 9.2 | 11.9 | 19.3 |

Data taken from Lobstein-Guth et al.[57]

**Table 4.** Specific optical rotations of ginkgolides and bilobalide

| Compound | $[\alpha]_D$ (°) | Temperature (°C) | Solvent | Concentration (g/100 mL) | Reference |
|---|---|---|---|---|---|
| G-A | −53.4 | 24 | Ethanol | 1 | 6 |
| G-A | −39 | ? | Dioxane | 1.0 | 1 |
| G-B | −52.6 | 24 | Ethanol | 1 | 6 |
| G-B | −52 | 24 | Ethanol | 1 | 35 |
| G-B | −63 | ? | Dioxane | 1.0 | 1 |
| G-C | −14.7 | 23 | Ethanol | 1 | 6 |
| G-C | −12.5 | 23 | Ethanol | 1 | 35 |
| G-C | −19 | ? | Dioxane | 1.0 | 1 |
| G-J | −2.5 | 20 | Dioxane | 1 | 12 |
| G-J | +1.6 | ? | Dioxane | 0.061 | 32 |
| G-K | +25 | ? | Acetonitrile | 1 | 37 |
| G-L | +40.7 | ? | Acetonitrile | 1 | 60 |
| G-M | −39 | ? | Dioxane | 1.0 | 1 |
| G-M | −36 | ? | Dioxane | 1.0 | 37 |
| BB | −64.3 | 20 | Acetone | 2 | 31 |

?, unknown.

**Table 5.** IR characteristics

| Wave number ($cm^{-1}$) | Transmission (%) | Assignment |
|---|---|---|
| *(a) IR characteristics of G-A (**1**) (nujol)* | | |
| 3546 | 35 | OH stretch |
| 3450 | 30 | OH stretch |
| 1798 | 20 | Lactone stretch |
| 1761 | 18 | Lactone stretch |
| 1759 | 15 | Lactone stretch |
| 1184 | 25 | C–O–C stretch |
| 1150 | 15 | C–O–C stretch |
| 1134 | 15 | C–O–C stretch |
| 1065 | 30 | C–OH stretch |
| 1044 | 25 | C–OH stretch |
| *(b) IR characteristics of G-B (**2**) (nujol)* | | |
| 3480[a] (shoulder) | 25 | OH stretch |
| 3446[a] (wide) | 15 | OH stretch |
| 1789 (broad) | 5 | Lactone stretch |
| 1774 (broad) | 5 | Lactone stretch |
| 1175 (broad) | 25 | C–O–C stretch |
| 1160 | 15 | C–O–C stretch |
| 1131 (broad) | 15 | C–O–C stretch |
| 1070 | 10 | C–OH stretch |
| 1043 | 25 | C–OH stretch |
| *(c) IR characteristics of G-C (**3**) (nujol)* | | |
| 3560 (sharp) | 30 | OH stretch |
| 3500 | 15 | OH stretch |
| 3400 (broad) | 15 | OH stretch |
| 1835 | 10 | Lactone stretch |
| 1789 | 5 | Lactone stretch |
| 1768 (shoulder) | 10 | Lactone stretch |
| 1165 | 20 | C–O–C stretch |
| 1140 | 10 | C–O–C stretch |
| 1080 (broad) | 10 | C–OH stretch |
| 1049 | 15 | C–OH stretch |

[a] In another paper by one of the authors only one OH stretch value of 3455 $cm^{-1}$ is given.[15]

could not be determined with IR spectroscopy. What could be observed is some intra-molecular hydrogen bonding in G–C (**3**). This induces a shift to higher wavelengths of the OH stretch (3560 $cm^{-1}$).[61]

Zhu et al. have performed a quantum chemistry calculation at the DFT-B3LYP/6-31G* level on the geometry and IR spectrum of G-B.[62a] The fully optimized geometry of G-B (**2**) was found to be consistent with an X-ray crystal structure. There was a good correspondence between the 156 predicted bands and the experimental IR spectrum. The O–H stretching bonds were located between 3501 and 3443 $cm^{-1}$ and the C=O stretching bonds between 1788 and 1764 $cm^{-1}$. The stretching of C–H bonds and the rocking of the *tert*-butyl group were found around 2900 and 1460 $cm^{-1}$, respectively. The bands below 1192 $cm^{-1}$ could not be assigned to any C–O stretching bond but are the result of the breathing of the condensed ring structure and other vibrations such as the rocking of C–H bonds.[62a] Recently, this group performed a new modelling structure on 117 ginkgolide derivatives to derive which parts of the molecule are involved in PAF-antagonism.[62b]

In Table 6 some additional IR data have been summarized.

### 4.5. Electron impact mass spectrometry (EI-MS)

Only once have EI-MS data been reported.[31] All spectra were obtained at 70 eV. Qualitatively, the fragmentation pattern for G-A (**1**), G-B (**2**) and G-C (**3**) is the same. The molecular ion can lose a methyl group, water, carbon monoxide, carbon dioxide and isobutene giving rise to fragments at $[M-15]^+$, $[M-18]^{+\cdot}$, $[M-28]^{+\cdot}$, $[M-44]^{+\cdot}$ and $[M-56]^{+\cdot}$, respectively. After the loss of $CO_2$, a further loss of the hydroxylactone ring is characteristic for the ginkgolides: ions at *m/z* 290, 306 and 322 for G-A, G-B and G-C, respectively.[31] In all the cases, the base peak is found at *m/z* 57 corresponding to the stable *tert*-butyl carbocation. The fragmentation pattern for G-A (**1**), G-B (**2**) and G-C (**3**) according to Weinges is schematically depicted in Figure 2 while that of BB (**8**) can be found in Figure 3. The mass spectrum of G-J (**4**) was given by Weinges et al., EI-MS: *m/z* (relative intensity): 424 $[M^{+\cdot}]$ (0.71), 409 (0.59), 396 (0.56), 380 (3.12), 378 (0.59), 362 (0.89), 350 (0.56), 57 (100).[12] For G-K, G-L and G-M no EI-MS have been published.

**Table 6.** Additional IR data of ginkgolides B, C, J, K and L, and bilobalide

| Compound | Matrix | Wave numbers ($cm^{-1}$) |
|---|---|---|
| G-B | KBr | 3520, 2950, 2860, 1780, 1630, 1480, 1450, 1353, 1135, 943, 793, 752[35] |
| G-C | KBr | 3580, 3520, 2990, 2925, 1785, 1630, 1410, 1173, 937, 892, 800[35] |
| G-J | ? | 3600, 3500, 3440 (all OH stretch)[15] |
| G-K | KBr | 1780[38] |
| G-K | ? | 3558, 3500, 3130, 1783, 1757, 1620[13] |
| G-L | Nujol | 1811, 1765[38] |
| G-L | ? | 3588, 3468, 3352, 1789, 1765[13] |
| BB | Nujol | 3450, 1820, 1810, 1775, 1210, 1150, 1005, 955[31] |

?, unknown.

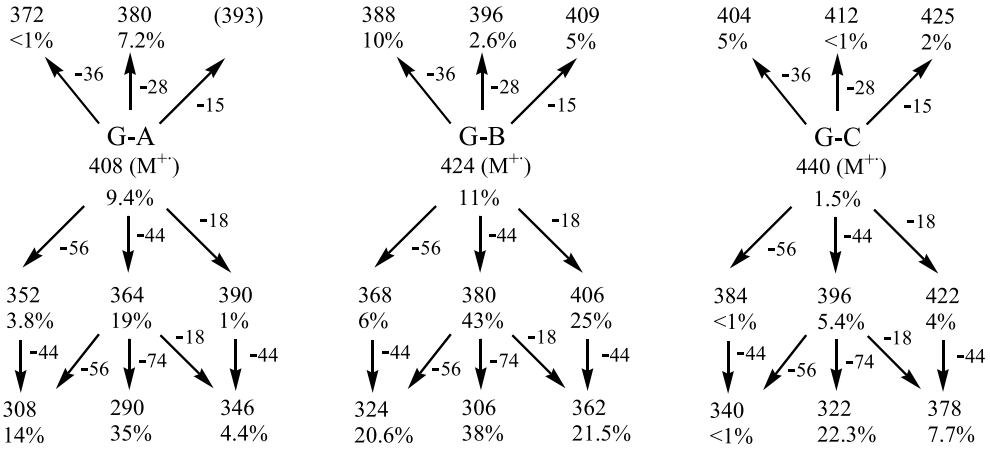



**Figure 2.** Mass spectrometric fragmentation pattern of ginkgolides A–C (**1**–**3**) under EI-MS (70 eV) conditions. Adapted from Weinges and Bähr.[31]

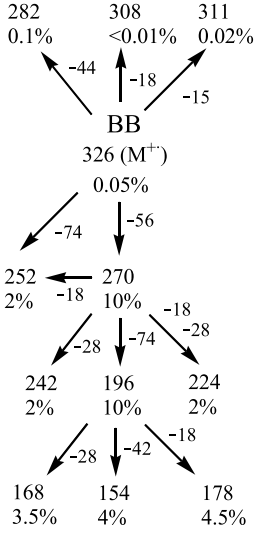



**Figure 3.** Mass spectrometric fragmentation pattern of bilobalide (**8**) under electron impact (70 eV) conditions. Adapted from Weinges and Bähr.[31]

### 4.6. Atmospheric pressure ionization mass spectrometry (API-MS)

During the last 12 years LC–MS has been frequently employed for the analysis of Ginkgo terpene trilactones, mostly for quantitative purposes. Initially a thermospray interface (TSP) was used.[63] Nowadays, atmospheric pressure chemical ionization (APCI)[64,65] and electrospray (ESI)[66,67] interfaces in either negative or positive mode are standard. These soft ionization techniques give very little fragmentation and are, therefore from a spectroscopic point of view, not very informative. Mostly, pseudomolecular ions are observed like $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$ and $[M-H]^-$. Mass spectra showing more fragmentation can be obtained by in-source fragmentation or by working in MS/MS mode (ion trap or triple quadrupole instruments). In the latter case, fragmentation arises through collision induced dissociation (CID). The best MS/MS study so far was carried out on a triple quadrupole working in negative mode with an ESI source and argon as collision gas at 10–15 eV collision energy.[67] The spectra have been summarized in Table 7. Very similar spectra—also obtained with negative mode ESI—have been published by Lu et al.[68,69] and Lang et al.[70] The mass spectral interpretation by Lu et al. appears erroneous at some points.

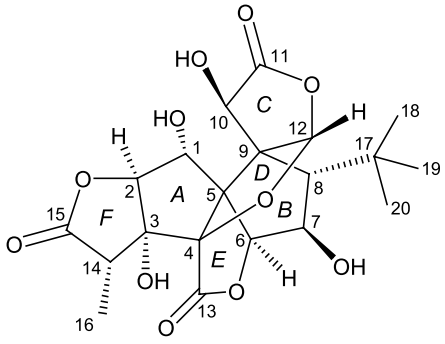
### 4.7. GC–MS data of trimethylsilyl ethers

After silylation in DMF with *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) at 120 °C, all Ginkgo terpene trilactones can be analysed by GC–MS. The 70 eV EI mass spectra of the TMS ethers of G-A (**1**), G-B (**2**) and G-C (**3**) have been published by Braquet without further discussion.[15,71] Unfortunately, the spectra published in that paper are too small to discern individual mass peaks. It is possible to observe that the base peak for all three compounds is the $[M-CH_3]^+$ and that further fragmentation is limited. The $[M-CH_3]^+$ is found at *m*/*z* 537, 625 and 713 for G-A, G-B and G-C. Better

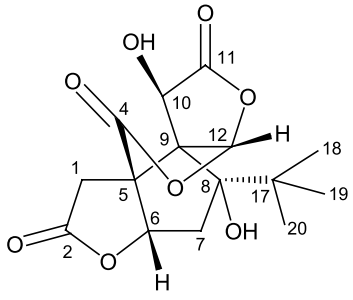
**Table 7.** Negative mode LC-ESI–MS/MS data of ginkgolides A–C and J, and bilobalide[67]

| Compound | *m/z* [fragment] (relative intensity) |
|---|---|
| G-A | 407 $[M-H]^-$ (100), 379 $[M-H-CO]^-$ (20), 363 $[M-H-CO_2]^-$ (20), 351 $[M-H-CO-CO]^-$ (90), 319 $[M-H-CO_2-CO_2]^-$ (10) |
| G-B | 423 $[M-H]^-$ (25), 395 $[M-H-CO]^-$ (10), 367 $[M-H-CO-CO]^-$ (100) |
| G-C | 439 $[M-H]^-$ (25), 411 $[M-H-CO]^-$ (10), 383 $[M-H-CO-CO]^-$ (100) |
| G-J | 423 $[M-H]^-$ (100), 395 $[M-H-CO]^-$ (3), 379 $[M-H-CO_2]^-$ (10), 367 $[M-H-CO-CO]^-$ (25) |
| BB | 325 $[M-H]^-$ (30), 281 $[M-H-CO_2]^-$ (2), 251 $[M-H-H_2O-C_4H_8]^-$ (30), 237 $[M-H-CO_2-CO_2]^-$ (25), 207 $[M-H-H_2O-C_4H_8-CO_2]^-$ (10), 193 $[M-H-CO_2-CO_2-CO_2]^-$ (25), 163 $[M-H-H_2O-C_4H_8-CO_2-CO_2]^-$ (100) |

spectra of the TMS ethers of BB, G-A, G-B and G-C were published by Tang et al.[72] and Deng and Zito[73] and are summarized in Table 8.




Numbering system for ginkgolide skeleton. A–F in italic are for designation of the six rings. The structure actually shown is that of G-C (**3**).




Numbering system for bilobalide (**8**).

### 4.8. NMR data

NMR data of Ginkgo terpene trilactones have been published by many different authors. The most complete or reliable studies on all ginkgolides are summarized in Tables 9–11. References to all NMR studies can be found in Table 12. Some of these studies contain incorrect structures or assignments and preference should be given to the data in Tables 9–11. Earlier studies suffer more from second order effects due to lower field strengths, making assignments more difficult. Additionally more recent NMR studies can confirm assignments by 2D experiments. Fewer studies have been carried out on BB (**8**). The NMR data of **8** are reported below.

$^1$H NMR bilobalide (**8**) (acetone-$d_6$, 250 MHz) $\delta$: 1.20 (H-18,19,20), 2.32 (H-7β, dd, $J = 13.6$, 7.0 Hz), 2.74 (H-7α, dd, $J = 13.6$, 7.0 Hz), 2.80 (H-1β, d, $J = 18.0$ Hz), 3.02 (H-1α, d, $J = 18.0$ Hz), 4.63 (OH-8, s, 1H), 4.99 (H-6, t, $J = 7.0$ Hz), 5.40 (H-10, d, $J = 4.3$ Hz), 6.36 (H-12, s), 6.38 (OH-10, d, $J = 4.3$ Hz).[27,81]

$^{13}$C NMR bilobalide (**8**) (acetone-$d_6$, 100 MHz) $\delta$: 27.0, 36.9, 38.2, 43.2, 59.0, 66.4, 69.7, 83.8, 87.4, 100.4, 173.4, 173.6, 178.3.[81]

NMR has also been used for quantitative studies of Ginkgo terpene trilactones in leaves and extracts. Such studies are based on the appearance of H-12 as a sharp singlet in a relatively non-crowded part of the NMR spectrum. The H-12 protons of bilobalide and ginkgolide A, B, C and J each resonate at a slightly different frequency and a careful integration of all H-12 signals and that of a singlet of a suitable internal standard in known concentration yields the absolute quantity of each terpene trilactone.[82–84]

### 4.9. X-ray crystallography

The structures of most Ginkgo terpene trilactones have been confirmed by X-ray studies. This method was already used by Sakabe et al. for the initial elucidation of the structure of G-A (**1**).[7] They used crystals of the 1-*p*-bromobenzoate derivative of G-A. The absolute configuration of G-A was determined by the method of Bijvoet, Peerdeman and van Bommel. No detailed X-ray data were presented in their paper.

Almost 20 years later a Belgian group published an X-ray study of G-B monohydrate (**2**). The crystals were formed by slow evaporation of a methanol–acetone–

**Table 8.** GC-EI–MS data of silylated ginkgolides A–C and J, and bilobalide

| Compound | *m/z* [fragment] (relative intensity) |
|---|---|
| G-A-$(TMS)_2$ | 537 $[M-CH_3]^+$ (100%), 493, 451, 391, 207, 187, 147, 113, 73[72,73] |
| G-B-$(TMS)_3$ | 625 $[M-CH_3]^+$ (80%), 581, 207, 147, 73 (100%)[72] |
| G-C-$(TMS)_4$ | 713 $[M-CH_3]^+$ (30%), 537, 207, 147, 73 (100%)[72] |
| BB-$(TMS)_2$ | 455 $[M-CH_3]^+$, 426, 398, 299 (100%), 269, 223, 179, 147, 73[72] |

**Table 9.** $^{1}$H NMR chemical shifts (ppm) and coupling constants (Hz) for ginkgolides A (**1**), B (**2**), C (**3**), J (**4**), L (**5**), K (**6**) and M (**7**)

| Solvent | $Me_2CO$ | DMSO | $Me_2CO$ | DMSO | $Me_2CO$ | DMSO | DMSO | DMSO | DMSO | DMSO |
|---|---|---|---|---|---|---|---|---|---|---|
| MHz | 600 | 400 | 600 | 400 | 600 | 400 | 300 | 300 | 200 | 200 |
| Reference | 74 | 75 | 74 | 75 | 74 | 75 | 12 | 37 | 76 | 37 |
| Compound | G-A | G-A | G-B | G-B | G-C | G-C | G-J | G-K | G-L | G-M |
| H-1α | 2.92 | 2.70[a] | — | — | — | — | 2.72 | — | 2.72 | — |
| H-1β | 2.06 | 1.76[a] | 4.29 | 4.03 | 4.24 | 4.00 | 1.81 | 3.84 | 1.53 | 4.13 |
| OH-1 | — | — | — | 4.89 | 4.80 | 4.98 | — | 5.17 | — | 4.83 |
| H-2 | 4.94 | 4.78 | 4.73 | 4.62 | 4.72 | 4.64 | 4.82 | 5.52 | 5.48 | 4.95 |
| H-3 | — | — | — | — | — | — | — | — | — | 2.95 |
| OH-3 | 5.30 | 6.33 | 5.54 | 6.45 | — | 6.48 | 6.27 | — | — | — |
| H-6 | 5.00 | 4.88 | 5.44 | 5.29 | 5.17 | 4.94 | 4.62 | 5.47 | 5.10 | 4.95 |
| H-7α | 2.30 | 1.99 | 2.18 | 1.92 | 4.37 | 4.07 | 4.20 | 2.19 | 2.09 | 4.00 |
| H-7β | 2.24 | 1.99 | 2.32 | 2.12 | — | — | — | 1.89 | 2.09 | — |
| OH-7 | — | — | — | — | — | 5.65 | 5.41 | — | — | 5.60 |
| H-8 | 2.00 | 1.65 | 2.01 | 1.71 | 1.86 | 1.57 | 1.57 | 1.89 | 1.86 | 1.61 |
| H-10 | 5.23 | 4.88 | 5.33 | 5.00 | 5.34 | 4.99 | 4.92 | 5.00 | 4.95 | 4.95 |
| OH-10 | 6.11 | 6.77 | — | 7.44 | — | 7.58 | 6.80 | 7.18 | 6.67 | 7.40 |
| H-12 | 6.12 | 5.96 | 6.17 | 6.05 | 6.19 | 6.09 | 6.03 | 6.06 | 6.04 | 6.10 |
| H-14 | 3.20 | 2.88 | 3.09 | 2.83 | 3.07 | 2.83 | 2.92 | — | — | 2.76 |
| H-16 | 1.32 | 1.06 | 1.31 | 1.08 | 1.33 | 1.13 | 1.13 | 1.91 | 1.93 | 1.17 |
| *t*-Bu | 1.21 | 0.96 | 1.22 | 1.01 | 1.31 | 1.10 | 1.10 | 1.04 | 1.03[60] | 1.09 |
| *J* (Hz) | | | | | | | | | | |
| *J* (1α-1β) | −15.2 | −15.2 | — | — | — | — | −15.2 | — | −13.4 | — |
| *J* (1α-2) | 7.3 | 7.7 | — | — | — | — | 7.8 | — | 7.1 | — |
| *J* (1β-2) | 8.9 | 7.7 | 8.0 | 7.4 | 7.2 | 7.0 | 7.8 | 7.9 | — | 7.0 |
| *J* (1β-OH-1) | — | — | — | — | — | — | — | 4.1 | — | 3.8 |
| *J* (2-3) | — | — | — | — | — | — | — | — | — | 8.7 |
| *J* (2-16) | — | — | — | — | — | — | — | 2.0 | 2.2 | 8.7 |
| *J* (3-14) | — | — | — | — | — | — | — | — | — | 10.7 |
| *J* (6-7α) | 4.2 | — | 4.3 | 4.0 | 4.2 | — | 4.0 | 3.0 | 3.4 | 4.4 |
| *J* (6-7β) | 0.8 | — | 0.3 | — | — | — | — | — | 0.0 | — |
| *J* (7α-7β) | −13.6 | — | −14.0 | −13.8 | — | — | — | — | — | — |
| *J* (7α-8) | 14.1 | 9.4 | 14.2 | 14.1 | 12.4 | 11.7 | 12.5 | — | 12.5 | 12.5 |
| *J* (7α-OH-7) | — | — | — | — | — | — | 6.6 | — | — | 6.4 |
| *J* (7β-8) | 4.9 | 9.4 | 4.8 | 4.1 | — | — | — | — | 6.6 | — |
| *J* (10-OH-10) | — | 5.2 | — | 5.6 | — | 3.9 | 5.0 | 5.4 | — | 5.4 |
| *J* (14-16) | 7.2 | 7.1 | 7.1 | 7.0 | 7.2 | 6.9 | 7.0 | — | — | 6.9 |

—, This denotes shift or *J* not present, not observable or not measurable.
[a] Assignment changed from original assignment.

**Table 10.** $^{13}$C NMR chemical shifts (ppm) for ginkgolides A (**1**), B (**2**), C (**3**), J (**4**), L (**5**), K (**6**) and M (**7**)

| Solvent | $Me_2CO$ | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO |
|---|---|---|---|---|---|---|---|---|
| MHz | 150 | 100 | 100 | 100 | 75 | ? | 50 | 50 |
| Reference | 74 | 75 | 75 | 75 | 77 | 13 | 76 | 37 |
| C | G-A | G-A | G-B | G-C | G-J | G-K | G-L | G-M |
| 1 | 37.9 | 37.1 | 73.8 | 73.8 | 36.6 | 74.1 | 37.5 | 77.0* |
| 2 | 88.9 | 87.9 | 91.8 | 92.0 | 87.7 | 85.3 | 80.3 | 85.4 |
| 3 | 87.9 | 86.3 | 82.9 | 83.1 | 86.0 | 155.2 | 160.4 | 48.8[#] |
| 4 | 101.7 | 100.5 | 98.5 | 98.4 | 100.1 | 92.3 | 92.8 | 94.8 |
| 5 | 69.7* | 68.3 | 71.7 | 66.6 | 63.2 | 76.4 | 72.4 | 67.0 |
| 6 | 86.8 | 85.5 | 78.6 | 79.2 | 85.9 | 80.9 | 86.9 | 79.9 |
| 7 | 37.5 | 36.5 | 36.6 | 74.2 | 73.4 | 35.6 | 35.0 | 73.4* |
| 8 | 50.3 | 48.8 | 48.6 | 49.2 | 49.3 | 48.8 | 48.6 | 48.9[#] |
| 9 | 68.5* | 67.0 | 67.4 | 63.9 | 62.6 | 69.2 | 68.2 | 64.4 |
| 10 | 70.5 | 68.9 | 69.0 | 69.1 | 68.6 | 69.0 | 69.0 | 68.9 |
| 11 | 174.9 | 174.5 | 174.0 | 174.0 | 174.3 | 174.1 | 173.3[a] | 173.9 |
| 12 | 111.4 | 109.7 | 109.6 | 109.7 | 109.5 | 109.1 | 109.6 | 109.5 |
| 13 | 171.9 | 171.1 | 170.3 | 170.8 | 171.1 | 169.2 | 169.1[a] | 174.0 |
| 14 | 42.1 | 40.6 | 41.5 | 41.7 | 40.5 | 125.1 | 124.1 | 36.0 |
| 15 | 177.4 | 176.8 | 176.4 | 176.6 | 176.6 | 173.4 | 174.0 | 177.3 |
| 16 | 8.8 | 8.4 | 7.9 | 8.1 | 8.3 | 8.8 | 8.5 | 14.8 |
| 17 | 33.3 | 32.1 | 32.0 | 32.1 | 32.5[12] | 32.0 | 31.7 | 31.9 |
| 18,19,20 | 29.8 | 29.0 | 28.9 | 29.1 | 29.0 | 28.8 | 28.6 | 28.9 |

?, unknown. *, [#] Assignments may be interchanged.
[a] Assignment changed from original assignment.

**Table 11.** $^{1}J$(C-H) coupling constants (Hz) for ginkgolide A–C[75] measured in DMSO-$d_6$ at 100.6 MHz

| | G-A | G-B | G-C |
|---|---|---|---|
| C-1/H-1α | 131.6 | — | — |
| C-1/H-1β | 131.6 | 141.8 | 141.8 |
| C-2/H-2 | 159.1 | 157.0 | 159.4 |
| C-6/H-6 | 172.0 | 166.5 | 168.0 |
| C-7/H-7α | 140.6 | 135.9 | 140.7 |
| C-7/H-7β | 129.0 | 125.4 | — |
| C-8/H-8 | 121.4 | 118.4 | 122.6 |
| C-10/H-10 | 140.0 | 142.1 | 141.4 |
| C-12/H-12 | 188.3 | 189.2 | 190.0 |
| C-14/H-14 | 126.0 | 127.2 | 122.6 |
| C-16/H-16 | 129.8 | 129.8 | 129.8 |
| C-18/H-18 | 125.3 | 124.3 | 126.7 |

**Table 12.** References to all NMR studies performed on Ginkgo terpene trilactones

| Compound | Type | References |
|---|---|---|
| G-A | $^{1}$H NMR | 6,8,9,31,35,38,74,75,77–79 |
| G-A | $^{13}$C NMR | 35,74,75,77,79 |
| G-B | $^{1}$H NMR | 6,8,35,38,74,75,77–79 |
| G-B | $^{13}$C NMR | 35,75,77,79 |
| G-C | $^{1}$H NMR | 6,8,35,38,74,75,77–79 |
| G-C | $^{13}$C NMR | 35,75,77,79 |
| G-J | $^{1}$H NMR | 12,77,79 |
| G-J | $^{13}$C NMR | 12,77,79 |
| G-K | $^{1}$H NMR | 13,37,38,60,78 |
| G-K | $^{13}$C NMR | 13,37,60 |
| G-L | $^{1}$H NMR | 38,60,76,78 |
| G-L | $^{13}$C NMR | 60,76 |
| G-M | $^{1}$H NMR | 37,38,60,78 |
| G-M | $^{13}$C NMR | 37,60 |
| BB | $^{1}$H NMR | 27,31,80,81 |
| BB | $^{13}$C NMR | 81 |

water solution. The crystal structure was solved by Patterson methods starting from Dreiding model data. The final reliability index $R$ was 0.089 for 3834 observed reflections collected with a four-circle diffractometer at 290 K. Bond lengths and fractional coordinates can be found in the original paper.[36] A comparison of a Dreiding model built with the help of the EUCLID programme with the X-ray structure showed a good fit, which was considered the result of the high rigidity of the molecule. Some torsional angles for rings A and B were published and compared with angles calculated from NMR studies. In general a good fit was found.[85] A view of G-B (**2**) is presented in Figure 4.

The same group has also published crystallograhic data of G-A monohydrate and G-C·ethanol·1.5 hydrate.[86] Crystals were prepared by slow evaporation of an ethanol/acetone/water solution. For G-A (**1**) and G-C (**3**) the final $R$ values were 0.042 (1399 observed reflections) and 0.074 (2964 observed reflections), respectively. Fractional coordinates of non-hydrogen atoms and bond lengths were reported. The X-ray studies of G-A (**1**), G-B (**2**) and G-C (**3**) by this group have been summarized by Dupont.[87]

Weinges et al. have published an X-ray study of bilobalide (**8**) and 10,12-di-*O*-pivaloyl-*iso*-bilobalide.[88] BB

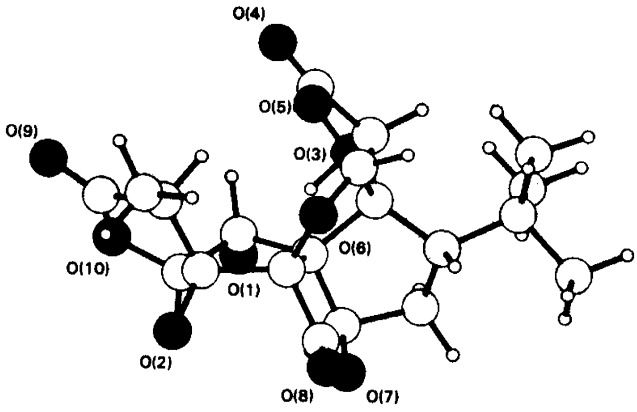



**Figure 4.** View of ginkgolide B monohydrate (**2**) as determined by Dupont et al. by means of X-ray crystallography.[36]

crystallized as a monohydrate. In the original paper, the length of all bonds and all atom coordinates can be found. This study confirmed the structure, which had been proposed 16 years earlier on the basis of chemical and spectroscopic evidence. The absolute configuration of BB was proven by the 'Exciton-chirality' method applied on the CD spectrum of 8,10-di-*O*-(*p*-bromobenzoyl)bilobalide. A view of BB (**8**) is given in Figure 5.

More recently, Zhao et al. carried out an X-ray study on G-A monohydrate, G-C sesquihydrate and G-J dihydrate, all at 120 K.[32] These low-temperature structures obtained from Mo-Kα data are more precise than the earlier room temperature determinations by a factor of 3 (vide supra). In the original paper, torsion angles and hydrogen-bonding geometries are presented. The improved data mostly confirmed the results of the earlier studies, including the absolute configuration. However, in contrast with the study of Sbit et al.[86] it was ascertained that in the persistent, unidirectional intramolecular hydrogen bond between the OH groups at C-1 and C-10 of G-C (**3**), the OH at C-1 functions exclusively

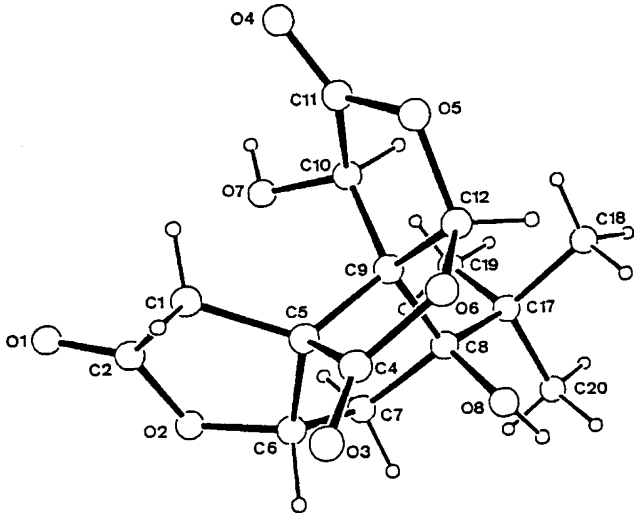



**Figure 5.** View of bilobalide monohydrate (**8**) as determined by Weinges et al. by means of X-ray crystallography.[88].

as donor. This can also be deduced from $^{1}H$ NMR data and is also true for G-B (**2**).[89] Like in the earlier studies of the Dupont group, it was found that the conformations of the B, C, D and E rings are very similar in all ginkgolides and that only in G-A (**1**) the A and F rings deviate. The earlier explanation by the Dupont group that this must be due to the absence of the OH group at C-1 in G-A, cannot be correct, however, as the conformation of the A and F rings in G-J (**4**) is similar to the conformation of those rings in G-B (**2**) and G-B (**3**) in spite of the fact that G-J like G-A lacks an OH group at C-1.[32] No alternative explanation was forwarded by Zhao et al. Views of G-A (**1**), G-C (**3**) and G-J (**4**) are given in Figure 6.

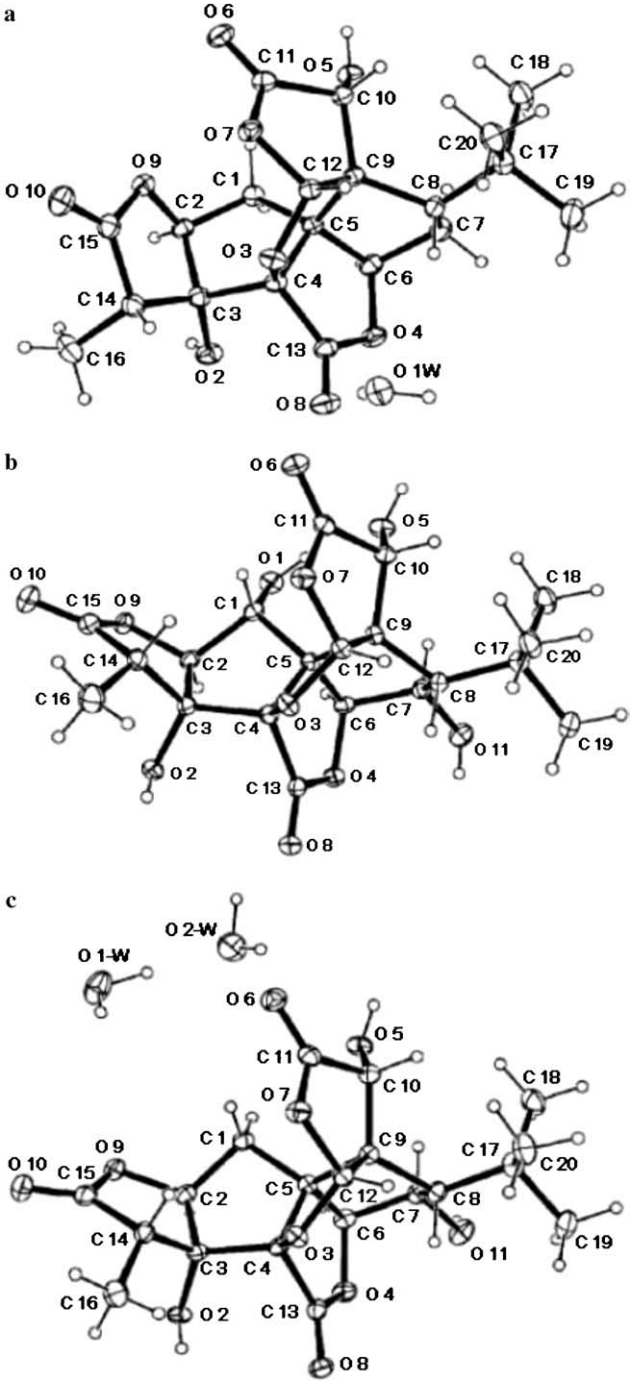



**Figure 6.** (a) View of ginkgolide A monohydrate (**1**) showing the atom-numbering scheme and ellipsoids at the 50% probability level. Modified from Zhao et al.[32] (b) View of one of the two independent molecules of ginkgolide C sesquihydrate (**3**) showing the atom-numbering scheme and ellipsoids at the 50% probability level. Modified from Zhao et al.[32] (c) View of ginkgolide J dihydrate (**4**) showing the atom-numbering scheme and ellipsoids at the 50% probability level. Modified from Zhao et al.[32]

During the last 20 years, there has been tremendous interest in the terpene trilactones from *Ginkgo biloba*, which has resulted in a more or less constant flow of 70 papers per year (Fig. 1a). This interest is mainly due to the interesting pharmacological properties of these compounds. Initially this was PAF-antagonism but the recent discovery of an antagonistic effect on glycine receptors[23–25] might give a new impulse. As there are considerable differences in biological activity between Ginkgo extracts and also between individual Ginkgo terpene trilactones, good knowledge about the identity and purity of used test substances is a prerequisite for a correct interpretation of pharmacological results. From all the data presented above, it is clear that ginkgolides and bilobalide can be well characterized. Thus, this compilation might facilitate further pharmacological and phytochemical studies on these unique compounds and possibly lead to the structure elucidation of new Ginkgo terpene trilactones.

## Acknowledgments

I wish to thank Professor Koji Nakanishi (New York) for making available to me two Ph.D. thesis describing many never published details about the early work of his group on ginkgolides, Professor Peishan Xie (Guangzhou) for providing me with copies of many Chinese articles, Professor Klaus Weinges (Heidelberg) for sending me the Ph.D. thesis of Matthias Rümmler, Professor Kurt Hostettmann (Lausanne) for sending me the Ph.D. thesis of F. F. Camponovo and Dr. Maarten Posthumus (Wageningen) for critically reading part of the manuscript.

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