The chemistry of fossils of *Ginkgo* and its ancestors

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Chemical analysis of organic solvent extractives of ten fossil-taxa (Gymnosperms) of the Paleozoic - Mesozoic eras indicate the chemical preservation of five main classes of naturally occurring compounds, viz. ginkgolic acids, terpenoidal lactones (ginkgolides A & B, bilobalide), biflavones (amentoflavone, bilobetin), procyanidins and anthocyanidins, in nine of them. The contents of these compounds are highest in *Ginkgo biloba*. The survival feat of *G. biloba* is remarkable. It is the last surviving representative of a race that, ages before the evolution of man, occupied a central position among the floras of the world. The preservation of the consortium of organic compounds and the longevity of *G. biloba* must in part be due to the potent antioxidant function of some of these compounds and the robust cage structures/molecular laminates of the metal ion complexes of the terpenoidal lactones and the lipophilic phenolic acids. A possible progenitor-progeny relationship between *Glossopteris* and *Ginkgo* was considered on the basis of similarities of their chemical constituents.

*Ginkgo biloba* Linn. (Ginkgoaceae), the maiden hair tree, fondly called by herbalists as the 'living fossil' is the last surviving representative of a race that, ages before the evolution of man, occupied a central position in the floras of the world. In early geological times the family Ginkgoaceae, which included several genera and a large number of species, was almost cosmopolitan and was represented in many parts of Gondwana. Also, possible *Ginkgo* - ancestors, e.g. *Glossopteris* and *Dadoxylon*, occurred in many parts of the world during the Permain period (300 - 250 million-year-present, mybp). As recently as the late tertiary period (ca. 1.6 mybp), relatives of *Ginkgo* existed in Spitzbergen and other high northern regions. Quite in contrast to its very recent confinement, in the wild state, to a small area in the eastern China, members of the *Ginkgo* ranged far and wide over the surface of the earth. The greatest lays in the past annals of *Ginkgo* and its projected immediate relatives, e.g. *Glossopteris* and *Dadoxylon* (Gymnosperms), seem to have transpired during the Mesozoic era (250 - 60 mybp). Leaf remains have turned up from geographically widely separated localities where Triassic (220 mybp), specially Jurassic (180 mybp), and to a lesser extent Cretaceous period, 120 - 60 mybp) *Ginkgo* flourished in the subpolar regions which were, in all probability, much warmer and more suited to comparatively luxuriant plant growth than at present.

The survival feat of *G. biloba* is indeed remarkable. It has not only survived for hundreds of millions of years of challenging geological and chemical onslaughts including the ice age on the surface of the earth, it is one of the very few living species that survived the man's most destructive act, the nuclear bombing at Hiroshima. The tree of *G. biloba*, at Hiroshima, that re-emerged after the devastation was normal and did not show any chromosomal aberration due to the nuclear radiation. This could be due, at least partly, to the strong antioxidant defence that *G. biloba* is known to possess. Plants existing in Perm-Carboniferous period (ca. 300 - 250 mybp), when the percentage of oxygen in the atmosphere was very high (>35%) and fluctuating had, obviously, enhanced antioxidant defence. It was, therefore, considered fascinating to study the chemical constituents of the different parts of fossil remains of *Ginkgo* and its possible ancestors, such as those belonging to *Glossopteridales*.

The plants included in the *Glossopteridales* are all extinct representative of a flora that once dominated the continent - Gondwana (which included the Indian Peninsula) during the Perm-Carboniferous period (Paleozoic era). By far the most common element of the flora is *Glossopteris*. There is evidence that the leaves of *Glossopteris* were borne on long and short shoots as in modern *Ginkgo* (Gymnosperm). The stems of *Glossopteris* are typical of Gymnosperms with abundant pycnoxylic wood and a pith surrounded by a ring of primary bundles. Despite these similarities, some other morpho-taxonomic
considerations raised controversies in respect of the projected ancestor-progeny relationship of Glossopteris and Ginkgo. Chemotaxonomic analysis was, therefore, warranted to settle this polemic. Again, beds where Glossopteridales are dominant, yield a great number of pyconoxic stems with wood of the Dadoxylon and Neomariopteris species (Gymnosperms). These two fossils also constituted the subject of the title study.

Ginkgophytes and Glossopteridales have received considerable attention from paleo botanical standpoint but their chemistry has so far remained unexplored. As a first step, we have studied the chemistry of ten fossils of Ginkgo, Glossopteris, Dadoxylon and Neomariopteris, of the early-Permian - Jurassic-Cretaceous periods (260 - 60 mybp), collected from different regions of India, England and USA (Table I).

Results and Discussion

The list of the test samples is given in Table I. The chemical analysis was focussed on five main classes of compounds, viz. ginkgolic acids, terpenoidal lactones, biflavones, procyanidins and anthocyanidins, occurring in the extinct (fossils) and extant (G. biloba) members of Ginkgo and related taxa. Additionally, the important metal ions (Fe, Cu, Zn) which constitute some crucial antioxidant defence and other enzyme systems in the living G. biloba, were analysed in the extinct and extant Ginkgo and in related fossils (Table II). The comparative analytical data of the chemical constituents of the fossils vis-à-vis living G. biloba are summarized in Table II.

The ginkgolic acids are represented by the generic structures 1a-f; the terpene lactones, viz. ginkgolid A 2a, ginkgolid-B 2b and bilobalide 2c; the biflavones, amentofoflavone 3a and bilobetin 3b; the procyanidins 4, stereochemistry undetermined; and the anthocyanidins, viz cyanidin 5a and delphinidin by 5b (Chart I). The separation and establishment of these compounds were accomplished by column chromatography, prep. TLC as comprehensive HPTLC, HPLC, GLC and GC-NMS analyses using authentic markers (for detection as well as for calibration of spectra for quantification).

The different parts of living G. biloba were rich in ginkgolic acids. Among the fossil-taxa also, G. biloba contained highest amount of these compounds Glossopteris and Dadoxylon also contained readily detectable amounts of ginkgolic acids, while Neomariopteris was devoid of these compounds (Table II).

The known natural occurrence of ginkgolic/anacardic acids has been limited to members of only three primitive families, viz. Anacardiaceae, Ginkgoaceae and Myristicaceae. For several reasons, however, the present detection of ginkgolic acids in the fossil-taxa should not be viewed as mere occurrence of phenolic lipids. Studies on biosynthesis of ginkgolic acids in G. biloba showed that the salicylic acid moiety was synthesized by polyketide pathway via malonic acid. The alkyl/alkenyl chains of ginkgolic acids were in different state and sub-cellular site of activation. Compounds and chains, e.g. malonic acid or palmitic acid, that were used for the biosynthesis of

<table>
<thead>
<tr>
<th>SI</th>
<th>Fossil No.</th>
<th>Genus/Species</th>
<th>Fossil part</th>
<th>Period of natural existence in mybp</th>
<th>Location of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Ginkgo biloba</td>
<td>I</td>
<td>Jurassic/200-180</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>G. biloba</td>
<td>I</td>
<td>Permian/280-240</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>G. biloba</td>
<td>p</td>
<td>&quot;</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>G. biloba</td>
<td>w</td>
<td>&quot;</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>G. digitata</td>
<td>I</td>
<td>Jurassic/120-60</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>G. huttonii</td>
<td>I</td>
<td>Permian/280-240</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Glossopteris sp. (PPL/Cu)</td>
<td>I</td>
<td>Permian/280-240</td>
<td>S</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Glossopteris sp.</td>
<td>st</td>
<td>&quot;</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>Dadoxylon sp.</td>
<td>w</td>
<td>&quot;</td>
<td>S</td>
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<tr>
<td>10</td>
<td>10</td>
<td>Neomariopteris sp. (Sc.Td. 131)</td>
<td>I</td>
<td>&quot;</td>
<td>S</td>
</tr>
</tbody>
</table>

I, leaf; p, petiole; st, stem; w, wood
A, Alaska, USA; R, Rajasthan (National Fossil Park), India; S, Soharjurdi Colliery, Bihar, India, Y, Yorkshire, England.
common lipids, were found to be very poor precursors for the side chain of ginkgolic acids. This significant complexity in the biosynthetic pathway of ginkgolic acids does seem to provide metabolic advantage to the producer organisms. The glycerol-3-phosphate dehydrogenase-inhibiting activity of ginkgolic acids provides the producer organisms protection against microbial infestation. The stability and selective onophoric properties of metal ion (Cu, Zn)-complexes of ginkgolic acids may explain their wide spectrum of biological activities.

Leaves and stems of living *G. biloba* contained higher amounts of ginkgolides than bilobalide. By contrast, the bilobalide contents of different parts of the fossil were higher than their ginkgolides content. The biosynthesis of the terpenoids originally involved an enzyme complex with an aspenine carrier protein (ICP), similar to that of the fatty acid synthetase. The latter catalyzes the production of C16 and C18 fatty acids from acetyl-CoA through with no smaller intermediates (e.g. C4, C6 acids) being formed. The evolutionarily most primitive terpenoid biosynthetic systems in prokaryotes were thus directed to higher polymers of tri-C30, tetra-C40, terpenes. Prokaryotes lack monoterpenoids, sesquiterpenes, and diterpenes. These major terpenoid biosynthetic targets in prokaryotes were modified luring the evolution of euukaryotes to release farnesyl pyrophosphate, from the ICP-complex, (leading to esquiterpenes) and geranyl-geranyl pyrophosphate (to produce diterpenes). The sesqui- and diterpenes thus produced interfered with the biosynthesis of triand tetramerpenes in competitors and predators, and/or detrimentally changed the permeability of their cell membranes. In other words, the sesqui- and diterpenes were evolved in eukaryotes for their ability to act as defensive agents. In addition to this, the presence of tertiary butyl substituent and the polylactone rings, with cage-structures, are two unique features of the terpenoids of *Ginkgo* and related species. Also, the extent and types of the oxygen functions in the ginkgolides and bilobalide, found in the *Ginkgo* and related fossils, suggest that these species in the remote past developed their sesqui- and diterpene biosynthetic pathways under a situation of hyperbaric oxygen pressure. Indeed, during that period, the atmospheric oxygen percentage was both high (> 3.5%) and fluctuating.

Flavonoids are characteristic of land plants and presumably evolved mainly during the Devonian period (350 mybp). The elaboration of the most primitive flavonoids, viz. the biflavones, in land plants, seemed to follow the malonoyl-coenzyme A pathways (leading to phenolic acids and lipids). It is argued that the initial function of biflavones (and equivalents) was that of a filter against UV irradiation and as internal regulatory agent, e.g. in redox reactions. Flavonoids often occur in higher concentrations and in greater structural diversity in the leaves (and stems) of plants than in the other parts.
Silyl Ginkgolates

1: Silyl Ginkgolates

2: Procyanidins

3: Amentoflavone, R = H
3 b: Bilobetin, R = CH₃

4: Procyanidins
(Stereochemistry at C₂,₃,₇,₁₇-undetermined)

5: Cyanidin, R = H
5 b: Delphinidin, R = OH

Chart 1—Structures of main chemical constituents of Ginkgo & related fossils

(Flowers, wood). Obviously, their role in the leaves must be different than in other parts of producer organisms. Consistent with this postulate, the higher abundance of the biflavonoids in the leaves and stems of the fossils (Table II) would seem to suggest their essentially protective role. The C - C linkages (3a,3b, Chart 1) are a hallmark of almost all 'primitive' flavonoids. The 'target' compound for the flavonoid biosynthesis in Ginkgo and in related fossil taxa was apigenin (the monomer of amentoflavone, 3a). The C₂, C₆-linkage of two apigenin moieties produced amentoflavone (3a), which on partial O - methylation produced bilobetin (3b). Surprisingly, the corresponding monomer (apigenin or its C₂ - O - methyl analogue) was absent in the fossil-taxa, analysed this study. The preservation of biflavones in fossils supports the postulate about the robustness of the biflavones. These compounds have stood the onslaughts of geological and chemical transformations for hundreds of million years of earth.

The woods and stems of Ginkgo, Glossopteris a Dadoxylon fossils contained highest concentrations of the procyanidins (4) and anthocyanidins (5a), (Table I). Neomarioperis also showed similarity the other fossil in respect of the occurrence
procyanidins and the anthocyanidins, but in lower concentrations. Procyanidins sparsely occurring in gymnosperms are believed to act as a constitutive defence factor.\(^{17,18}\) They are reported to be potent antioxidants, anti-mutagenic and radiation protectors.\(^{18}\)

The fossils (Table I) showed remarkable consistency in respect of their metal ion contents (Table II). These metal ions are the essential constituents of a number of vital enzymes in eukaryotic cells.\(^{5}\)

**Conclusion**

Only a few studies were reported in the past four decades on the chemistry of preserved remnants of organic compounds in fossil-taxa.\(^{19,22}\) It is too soon to comment what the practical significance of the earlier studies could be in tracing the role of the detected chemical in the stability and metabolism of the extant species. The present study, on the other hand, has demonstrated that fossils have been found in which the degradative processes have not been too severe and/or their robust chemical compounds have been competent to withstand the onslaughts of nature for hundreds of million years. The comparative chemical profiles of *Ginkgo* and related taxa (extinct and extant species) have established this point. This study has shown, for the first time, striking similarities between *Ginkgo* and *Glossopteris/Didaxylon* fossils, in respect of their chemical constituents, and thereby indicated possibility of their progeny-progenitor relationship, a subject which hitherto remained polemical.

**Experimental Section**

**Test samples**

The details of the test samples, their genus/species, fossil parts analysed, their age, and location of collection, are summarized in Table I.

**Techniques**

**HPTLC** — CAMAG TLC (Plate material, silica gel 60 F\(_{254}\)) evaluation assembly (CATS integration, V.4.05) was employed. The detection of spots was done by both quenching and fluorescence modes using authentic markers. Prior heating of the developed plates, at 110°C for 20 min, was necessary to detect the ginkgolides and bilobalide spots. Three solvent systems, viz., chloroform-methanol (90:10) (solvent-1), toluene - ethyl acetate - acetone - hexane (4:3:2:1) (solvent-2), and ethyl acetate - formic acid - acetic acid - water (100:11:11:27) (solvent-3), were routinely used as developers for the three major classes of compounds, viz. ginkgolide acids, terpenoidal lactones, and biflavonoids, respectively. For the separation of ginkgolide acids from other common lipids by prep. TLC, chloroform - methanol - 10 M NH\(_4\)OH (65:30:5) was used.

**HPLC** — Waters Associate HPLC assembly (Millennium 2.10) with a RP C-18 column, equipped with a PDA (Model 996) detector was employed. Two mobile phase, viz. methanol - water - acetic acid (85:15:0.2) (solvent-4) and acetonitrile - water - phosphoric acid (80:20:0.1) (solvent-5), were used. Additionally, for the analysis of biflavonoids, in the fossil leaf extracts, a previously described method\(^{2,3}\), used for the detection and quantification of biflavones in the leaf extracts of fresh *G. biloba*, was followed. This method involved a LichroSorb - Diol column and a ternary elution system, - hexane - chloroform (25:75) - tetrahydrofuran (100) (solvent - 6), the biflavones were detected at 350 nm. Authentic markers were used for setting the calibration curves and establishing the identity of the compounds.

**GLC** — A Hewlett-Packard (HP-5890) instrument, equipped with a flame-ionization detector (FID), coupled to a microprocessor controlled integrator (HP-3394A) was employed. Glass column (1.8 m x 0.2 cm i.d) packed with a 3% SE - 30 non-polar liquid phase coated on chromosorb - W (HP) (80 - 120 mesh) was used for the analyses of the silylated terpenoidal lactones. The conditions employed were - oven 210°C (2 min hold), 6°C/min increase upto 320°C (15 min hold), FID, 380°C; carrier gas, N\(_2\), 30 mL/min. To substantiate the findings, a previously described GC-FID method\(^{24}\) for the analysis of ginkgolides and bilobalide in the extracts, was also applied.

**GC-MS** — Gas chromatographic separation was performed on a DB-5 (30 m x 0.25mm i.d.) capillary column, oven temperature at 80°C (2 min hold) to 250°C (30 min hold) at 10°C/min rise; injection temperature was 250°C. MS data were obtained from a Varian 3800/Saturn 2000 model instrument, operating at an ionization potential of 20 eV.

**Electron Probe Micro-Analyzer (EPMA)** — The analysis of metal ions in the fossil-wood marc (Scheme II) was performed on a Shimadzu EPMA - 8705 instrument. The conditions were ACC.V (kV) 20.0; S.C. (micro -), 0.20; beam size, 30 - 159 μm.
Marker samples

Ginkgolides, bilobalide and ginkgolic acid mixture were obtained from Dr. Willmar Schwabe GmbH, Karlsruhe, Germany; the biflavonoids were isolated from G. biloba (fresh leaves) following a published procedure. Treatment of fossil samples — The general procedure followed for the separation of the fragile parts (leaf, stem, petiole) of fossils, from mineral matrix, and isolation of their chemical constituents is depicted in Scheme I. A different procedure was followed for the extraction and separation of chemical constituents from the fossil-wood(s) (Scheme II).

The first step was to disentangle the specimen, e.g. the whole or part of the fragile organ (leaf, stem, petiole) from the mineral matrix. This was carefully carried out by physical/mechanical, rather than chemical, means in order to avoid possible chemical alteration of the secondary metabolites (Scheme I).

Treatment of fractions

Fractions f1-f5 — Ginkgolic acids (GA), occurring in these two fractions, (f1/f2), were separated from common lipids by prep. TLC on silica gel (E. Merck). Samples of 10 mg, in chloroform, were streaked on 20 × 20 cm plates (1 mm thickness) a developed in chloroform - methanol - 10 M NH₄Cl (See techniques). The band of GA appearing around Rₚ 0.65 was detected by the blue fluorescence under UV-B light. TLC scrapings of the GA - band were eluted with diethyl ether, saturated with conc. HCl. The extract was washed with water to neutrality, and the solvent was removed under reduced pressure. The remaining portion of the residue was subjected to HPTLC (solvent-1), Rₚ 0.28; λmax, reflectance 243, 302 nm; HPLC (solvent-5), tR 2.12 min (main peak); λm: PDA 240, 290 nm. Authentic ginkgolic acid mixture was used to substantiate the identity of the compound. The remaining portion of the mixture of ginkgol...
G. biloba fossil wood-powder (110 g)

Marc tested for metal ions (EPMA)

Methanol extract

evapd./red. press.

Residue (534 mg)

suspended in water

Aq. suspension

Benzene extract

Solvent evapd.

gummy residue (52 mg), Fraction-f5
(processed for ginkgolic acids)

ppt. (41 mg), Fraction-f6
(mixture of terpene lactones)

Chloroform extract

evapd.

Residue (68 mg), Fraction-f7
(biflavonoids) (HPLC)

Ag. suspension

Chloroform

extract

evapd.

Residue, fraction-f6
extracted with chloroform

Ag. layer

evapd.

Residue, fraction-f4
procyanidins cyanidin & delphinidin
(HPTLC prep. TLC, UV)

Ginkgo fossil wood - powder (110 g)

extraction/Soxhlet, methanol, 30 h.

Acids was silylated. The mixture of ginkgolic acids (GA) (0.5 mg), was dissolved in pyridine (0.2 mL) and treated with hexamethyl disilane-trimethyl chlorosilane (2:1, 0.1 mL) to form the corresponding silylginkgolates (OTMS derivatives). GC-MS analysis of the OTMS derivatives was carried out, under the conditions mentioned before (See techniques) and the results are summarized in Table III. The OTMS derivatives prepared from the fraction-f1 of Veomaripteris (leaf fossil) did not show the presence of ginkgolates.

Scheme II—Extraction of chemical constituents from fossil-wood

The relative percentage composition of the C6-alkyl/alkenyl chains of ginkgolic acids in the fossil and in fresh G. biloba (leaf) extracts was determined after catalytic hydrogenation of the mixture of ginkgolic acids. This was done by using Pd-C (10%), in methanol, at 35-lbs pressure of hydrogen. The product with different alkyl side chains was analysed by HPLC (solvent-4) and GC. The results are recorded in Table IV.

Fractions f2/f3/f4—The mixture of terpenoidal lactones (TLa) and biflavonoids (BFb), contained in
fraction-f₂, was separated by column chromatography over celite using chloroform and chloroform - ethanol (traces) as the eluents. The later fractions of eluates were evaporated to give a mixture of TLₗ (HPTLC, see below)). The identities of the compounds were established by GC. A portion of the residue (0.5 mg) was dissolved in pyridine (0.2 mL) and treated with a mixture of trimethylchlorosilane (TMCS, 1%) and N, O-bis-trimethylsilyltrifluoroacetamide (BSTFA, 0.1 mL). The mixture was warmed at 60°C for 30 min, cooled to room temperature and then injected to GC column. An internal standard of squalene, in chloroform-methanol (1:1), was used for the calibration. Authentic markers were used, under similar conditions, to substantiate the identification. The respective peaks appeared at : 29.2 (bilobalide), 42.1 (ginkgolide A), 43.5 min (ginkgolide B), and 35.5 min. (Squalene).

The early fractions from the column chromatographic run contained biflavonoids. The mixture was subjected to HPLC using solvent-6 as the eluent. Amentoflavone (3a), tₚ 45.4, and bilobetin (3b), tₚ 42.6 min were detected and quantified in all the fossil samples (including Neomarikopteris (2 samples). Additionally, G.biloba (leaf)-fossils (2 samples) showed the presence of isoginkgetin (str.3, C₇-ΟCH₃), tₚ 36.0 min. Fraction-f₁ from fresh G.biloba (leaf) showed the presence of all the four earlier reported²³ biflavonoids, viz. Schidopitysin, ginkgetin, iso-ginkgetin, bilobetin, and also amentoflavone (3a). Likewise, treatment of fractions-f₁ on HPTLC and HPLC showed the presence of these biflavonoids.

HPTLC (solvent-3)-Amentoflavone, Rf 0.26; λmax, reflectance 270, 338 nm; bilobetin, Rf 0.32; 270, 334 nm; isoginkgetin, 0.40; 271, 330 nm.

The residue from fraction-f₆ (Scheme II) on HPTLC and GLC (as above) showed the presence and quantities of TLₗ (Table II).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Mol. ion peak (m/z)</th>
<th>Fragment ion peaks m/z (rel. abundance %)</th>
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<tbody>
<tr>
<td>a</td>
<td>464 (2)</td>
<td>449(100), 374 (7), 359 (9), 293 (5), 219 (42), 205 (8), 73 (70).</td>
</tr>
<tr>
<td>b</td>
<td>462 (7)</td>
<td>447 (100), 372 (20), 357 (14), 293 (4), 219 (36), 205 (4).</td>
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<tr>
<td>c</td>
<td>492 (3)</td>
<td>477 (100), 402 (12), 387 (9), 219 (38), 205 (11), 91 (22), 73 (22).</td>
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<tr>
<td>d</td>
<td>490 (5)</td>
<td>475 (100), 400 (12), 385 (8), 293 (5), 219 (34), 205 (5), 91 (20), 75 (7).</td>
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<tr>
<td>e</td>
<td>518 (5)</td>
<td>503 (100), 428 (12), 413 (6), 293 (5), 219 (35), 205 (4), 93 (7).</td>
</tr>
<tr>
<td>f</td>
<td>516 (3)</td>
<td>501 (100), 426 (18), 411 (11), 293 (5), 205 (5), 90 (7), 75 (8).</td>
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</table>

Table III — Mass spectral fragmentation patterns of silyl-ginkgelates*, isolated from Ginkgo and related fossils

* see Chart 1

Table IV — Composition of hydrogenated ginkgolic acids (Gₗ) in the leaves of G.biloba (living) and in the fossils leaves²⁴

<table>
<thead>
<tr>
<th>Structure</th>
<th>Relative % of GA by</th>
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<tr>
<td>C₆-Side chain</td>
<td>GC</td>
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<tr>
<td>C₁₄ H₂₇</td>
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</tr>
<tr>
<td>C₁₄ H₃₁</td>
<td>61-72</td>
</tr>
<tr>
<td>C₁₇ H₃₉</td>
<td>29-35</td>
</tr>
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</table>

*a. G.biloba (3 samples), G.digita, G.huttonii, Glossopteris; b. Dadoxylol (leaf) contained only traces of GA; Neomarikopteris was free from GA. Note - The inherent similarities in the composition of the C₆-alkylated ginkgolic acids preserved in the extant and extinct Ginkgo species and in the possible ancestor (Glossopteris).
using cyanidin and delphinidin (chloride) as markers. Cyanidin, Rr 0.26, λmax 277, 534 nm; delphinidin, Rr 0.15; λmax 275, 542 nm. The prep. TLC scrapings of the corresponding zones, on usual processing, afforded the two compounds, whose identities were confirmed by direct comparison with authentic markers.

Acknowledgements
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References