THE GINKGOLIDES†

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I. INTRODUCTION

The ginkgo tree (*Ginkgo biloba;* Icho in Japanese) occupies a unique position in botany for several reasons. It is the sole representative of its family and is not linked to any other living plant; its origin extends as far back as the Paleozoic. The order *Ginkgoales* was once widely distributed throughout the world but in the last few million years all species excepting *Ginkgo biloba* have become extinct, the other species being found only as fossils in petrified woods; moreover, *Ginkgo biloba* itself is believed to have remained unchanged for the last million years or so. The ginkgo tree is thus called a "living fossil" or "fossil tree". The ginkgo tree was unknown outside the Orient before the 18th century but is now rather commonly distributed in Europe, America and other continents.

The ginkgo tree has already been subject to numerous chemical investigations, which have led to the characterization of the following compounds: the phenolic compounds, ginkgol[1,2], bilobol[3] and ginkgolic acid[1] from the fruit; cyanogenetic glycosides and amino acids from the seeds[4,5]; the aliphatic compounds, ginnol[6], ginnon[7], and *n*-hexenal[8], and shikimic acid[9] from the leaves; the bisflavone ginkgetin[10–12] from the leaves; and finally D-sesamin[13] and the sesquiterpenoid bilobanone[14] from the heartwood.

Study of the root itself was commenced in this laboratory in 1960 under the late Professor S. Fujise, but the early stage of the work was seriously hindered by purification problems and polymorphism.

† Shortly after the Symposium, it was discovered that dehydroginkgolide A (*Figure 13*) very easily underwent a photochemical rearrangement and that several properties of the rearrangement product had in fact been wrongly attributed to dehydroginkgolide A. In particular, the lack of a Cotton effect in a sample of dehydroginkgolide A (actually photodehydroginkgolide A which had been formed during storage) indicated the absence of an α-hydroxylactone function in the original ginkgolides, and thus led us wrongly to believe that a hemiacetal function was present in these substances.

Once the occurrence of this photochemical rearrangement had been recognized, the existence of an α-hydroxylactone in the ginkgolides became apparent; moreover, the ease with which this rearrangement occurred provided important evidence in the derivation of the current structure of the molecule.

The present content has been modified from the lecture given in Stockholm, since an article based on a wrong structure would not be of much significance. (September 1966).

	R₁	R₂	R₃
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide M	H	OH	OH
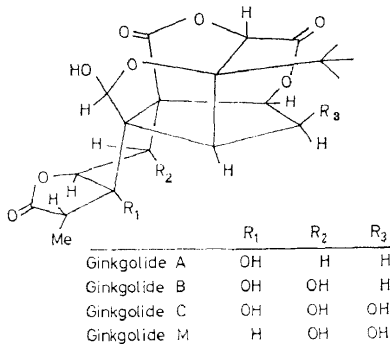
Structure of Ginkgolides proposed at Stockholm (July 1966).

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For structural studies of cage molecules such as the ginkgolides, molecular models are indispensable to clarify the spatial relation of the various groups. This was especially so in the present case because the partial structures (described later in the article) were connected by intervening quaternary carbon atoms, and because the only carbocyclic ring turned out to be a spiro[4.4]nonane system, which became apparent only in the final stages of the structure elucidation. Anisotropic effects displayed in the n.m.r. spectra, especially the difference in chemical shifts when one group is changed to another (e.g., oxidation of hydroxyl to ketone), the nuclear Overhauser effect, the photochemical rearrangement, and other spatial interactions, as studied with molecular models, were of extreme importance in determining the structure. However, since such an argument would be difficult to present without the extensive use of models, an attempt has been made to derive the structure as much as possible without recourse to spatial considerations. The derivation presented in the following therefore differs slightly from that actually used in the structure determination but on the whole follows the same logical sequence.

Several ginkgolide derivatives, with and without heavy atoms, had been sent to Professors M. Kakudo and Y. Sasada, Osaka University, and Professor W. N. Lipscomb and Dr. J. W. Moncrief, Harvard University, for x-ray analysis, but the crystals sent have not been easily amenable to this powerful technique, probably owing to our inappropriate recrystallization techniques or deterioration of crystals in the post. Nevertheless, we are grateful to these workers for their interest and preliminary examinations of the ginkgolides. On the other hand, it has been particularly gratifying to us to be informed, after completion of our studies, that the entire structure including the absolute configuration had been confirmed by a totally independent x-ray study (private communication from Dr. N. Sakabe, Nagoya University).

The structure elucidation based entirely on chemical reactions and spectroscopic methods has led to the disclosure of many fascinating chemical and physico-chemical phenomena, and has contributed greatly to increase our appreciation of the behaviour of complex organic molecules.

II. ISOLATION OF GINKGOLIDES

The procedure being followed at present is outlined in *Figure 1*. Four compounds designated ginkgolides A, B, C, and M (for minor) have been isolated by this procedure. A methanol extract of the undried chopped root bark was concentrated to a syrup and extracted with benzene. The solid material which precipitated from the aqueous layer crystallized from ethanol as mixed crystals of the ginkgolides. A solution of these crystals in acetone was absorbed on Celite, the Celite was placed as a layer on top of a silica gel column, and the column was eluted with chloroform containing traces of acetone and ethanol. This afforded a ginkgolide A–ginkgolide B mixture, a small amount of ginkgolide M and finally ginkgolide C. The separation of ginkgolides A and B was extremely tedious, and satisfactory results were achieved only after a 10–15 step fractional crystallization procedure, the purity being checked either by n.m.r. or optical rotation. Furthermore,
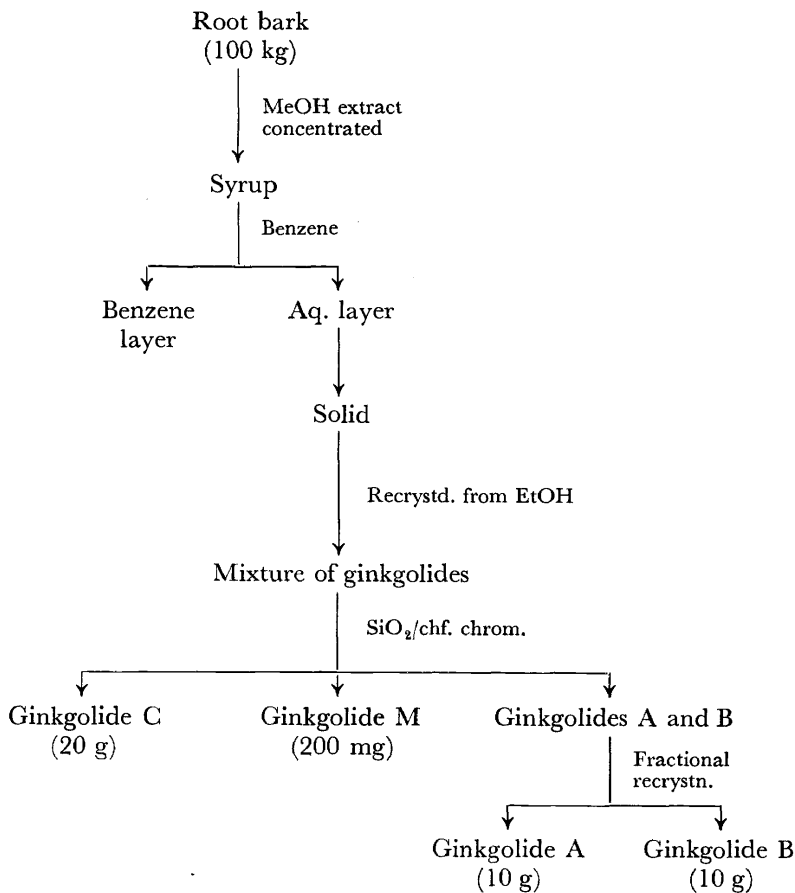
Root bark
(100 kg)

| MeOH extract
| concentrated
↓

Syrup

| Benzene
|

Benzene
layer   Aq. layer

|
↓

Solid

|
| Recrystd. from EtOH
↓

Mixture of ginkgolides

| SiO_2/chf. chrom.

Ginkgolide C   Ginkgolide M   Ginkgolides A and B
(20 g)   (200 mg)
                                        | Fractional
                                        | recrystn.
                          ↓   ↓
                    Ginkgolide A   Ginkgolide B
                    (10 g)   (10 g)

Figure 1. Scheme for the isolation of ginkgolides from *Ginkgo biloba* L. (*Ginkgoaceae*) (five trees of 30 cm in diameter were used)

separation was complicated by the strong tendency of ginkgolide A and especially ginkgolide C to exhibit polymorphism.

III. STRUCTURE OF THE GINKGOLIDES

(a) Molecular formulae and general properties

The ginkgolides are C_{20} compounds but owing to the easy fragmentation of their derivatives upon electron impact, the molecular formulae could be established only with the aid of high resolution mass spectrometry using a direct inlet system. Ginkgolide A dimethyl ether gave a clean molecular ion peak at 436·168 (calc. for $C_{22}H_{28}O_9$, 436·173). All the ginkgolides are bitter in taste, and are extremely stable towards mineral acid such as conc. HNO_3, conc. HCl, warm conc. H_2SO_4, as well as to warm 1N NaOH. Evaporation to dryness of a conc. nitric acid solution of the ginkgolides results in recovery

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of crystalline starting material. The molecular formulae and specific rotation of the ginkgolides are given in *Table 1*.

Table 1. Molecular formulae and specific rotation of ginkgolides

Compound	Mol. formula	Sp. rotation $[\alpha]_D$
Ginkgolide A dimethyl ether	$C_{22}H_{28}O_9$†	—
Ginkgolide A	$C_{20}H_{24}O_9$	$-39°$
		$(c, 1\cdot0, \text{dioxane})$
Ginkgolide B	$C_{20}H_{24}O_{10}$	$-63°$
Ginkgolide C	$C_{20}H_{24}O_{11}$	$-19°$
Ginkgolide M	$C_{20}H_{24}O_{10}$	$-39°$

† Found: mol. wt. 436·168. $C_{22}H_{28}O_9$ requires mol. wt. 436·173.

(b) Functional groups

The absence of any ketone grouping in the ginkgolides is supported by the plain negative optical rotatory dispersion (o.r.d.) curves exhibiting no Cotton effect in the range 250–700 mμ. The number and nature of the hydroxyl groups were deduced by the conventional n.m.r. techniques of comparing the spectra of the original ginkgolides with those of the acetates and by measurements of n.m.r. spectra in DMSO–d$_6$ and addition of D$_2$O or deuterated acid. The results are summed up in *Table 2*. Because of solubility problems, the n.m.r. spectra were in general measured as trifluoroacetic acid solutions: in general, the proton chemical shifts (apart from hydroxyl protons) were within *ca.* $\pm$ 0·2 p.p.m. of their positions when measurements were made, when possible, in deuterochloroform. Unless stated otherwise, the chemical shifts refer to trifluoroacetic acid solutions.

Table 2. Studies on the functional groups present in ginkgolides

KETONE: None, plain optical rotatory dispersion
HYDROXYL: From acetate and DMSO–d$_6$ n.m.r. spectra

	2°	3°
Ginkgolide A	one	one
Ginkgolide B	two	one
Ginkgolide C	three	one
Ginkgolide M	three	—

t-BUTYL:
  N.m.r. Spectra : 9 H(s) at 1·2–1·3 p.p.m. in trifluoroacetic acid
  Mass Spectra : Peak at 57·074 (50–100%). Calc. mol. wt. for C_4H_9, 57·070
              M–57 peak in ginkgolide A-LiAlH$_4$ reduction product
   (triether) at 309 (31%)
  Kuhn–Roth oxidation
    of Ginkgolide C: *t*-BuCOOH (*p*-bromophenacyl ester)

The n.m.r. spectra of all derivatives, excepting photodehydro-ginkgolide A, showed a 9-proton singlet in the range 1·2–1·3 p.p.m. Since it is most

unlikely that three methyl groups attached to different carbon atoms would constantly be subject to identical overall anisotropic and electronic effects, this conspicuous signal suggested the presence of a *tert*-butyl group, for which no precedence has been recorded in the natural products field. Moreover,
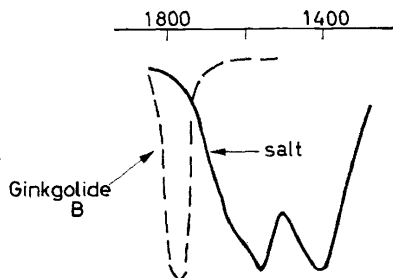
I.r. spectra of ginkgolide B (KBr disk) and that of its sodium salt (in KBr/NaOH disk)

the mass spectra had a strong peak (relative intensity 50–100 per cent of base peak) at 57·074, which corresponded to the stable *tert*-butyl cation, 57·070. This unique characteristic of the ginkgolides, *i.e.*, presence of the *tert*-butyl group, was finally established by isolation of pivalic acid, characterized as the crystalline *p*-bromophenacyl ester, by oxidation of ginkgolide C under Kuhn–Roth conditions.

The i.r. spectra (KBr, acetonitrile, dioxane) of the ginkgolides show a strong but ill-defined absorption around 1790 cm^{-1}. This band was replaced by carboxylate absorptions when, for example, an aqueous sodium hydroxide
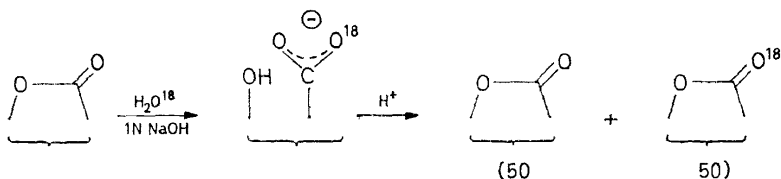
H_2O containing 30% O^{18}
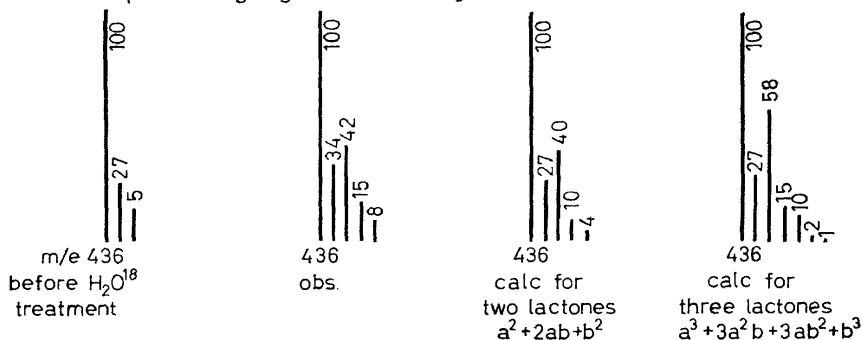
M^+ peaks of ginkgolide A dimethylether (mol. wt. 436)

Figure 2. Attempts to establish the number of lactone rings from the mass spectrum of the H_2O^{18}-treated compound

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solution of ginkgolide B was evaporated to dryness at room temperature under reduced pressure, and the residual mixture of the sodium salt and sodium hydroxide was made up into KBr disks; ginkgolide B was recovered upon dissolution of the KBr disk and acidification, thus indicating no chemical change excepting cleavage and recyclization of the lactone rings. This indicated that all three lactones are *essentially strain-free*, and further that the ginkgolides contained no ketonic carbonyl group.

The exact number of lactones had remained a problem for a long period. The first clear experimental evidence for the presence of three lactones was secured through the detailed n.m.r. analysis (cf. para. IIIk) of "ginkgolide A triether" which showed that three carbonyl groups had been reduced to methylene groups. Accordingly, a modified technique for lactone titration was developed (*Table 3*) and the results fully confirmed the conclusions drawn from the n.m.r. analysis. Attempts to derive the number of lactones by mass spectrometric measurements of ginkgolide A dimethyl ether utilizing H_2O^{18} (*Figure 2*) led to anomalous results, which on the other hand, were of use in clarifying the chemical behaviour of the lactone groups.

The n.m.r. spectra of the ginkgolides and derivatives were characterized by two lowfield singlets, I and J (cf. para VI) generally appearing at *ca.* 5·4 and 6·2 p.p.m. (trifluoroacetic acid), respectively. The two signals can be easily differentiated because signal J was invariably taller than signal I. The broader singlet I is due to the carbinyl proton of the secondary hydroxyl, which together with one of the lactones, constitutes an α-hydroxylactone, $-CH_I(OH)-CO-O-$ (cf. para IIIg).
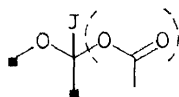
Lactones : three

i.r. 1780 cm^{-1} (Mecn, dioxane)
n.m.r. of ginkgolide A-triether

Potentiometric titration
Mass spectrometry of
  ginkgolide A-dimethylether
  (O^{16} and O^{18})

Ether

J at 6·17 p.p.m. (sharp s., CF_3COOH)
in ginkgolide A
(■ indicates carbon with no proton)

As regards the other lowfield singlet J, which appears as a singlet even in DMSO–d_6, we shall only mention at this stage that it is flanked by an ethereal oxygen and one of the lactone groups as shown above. The nine oxygen atoms in ginkgolide A are thus accounted for by three lactones, two hydroxyls, and one ether group.

(c) Number of lactone rings

A large number of potentiometric lactone titrations were carried out since in spite of the fact that conventional titrations (Method A) seemed to indicate that only two lactones were present, n.m.r. analyses of the triether and absence of other functions to account for the two remaining oxygen atoms suggested there should be three lactones. The objective of establishing the presence of three lactone rings was finally achieved by Method B in which the alkali solutions were evaporated to dryness; the i.r. spectra of the residual mixture of sodium hydroxide and the sample (KBr disk) showed that all carbonyl functions had been cleaved to carboxylate groups, and moreover no rearrangement had occurred by this treatment because unchanged starting materials were recovered upon acidification of the residues. Apparently, one lactone ring remains unopened under conditions of Method A.

Table 3. Potentiometric titration

(One lactone grouping is more hindered than the others. All pKs are below 6)

Compound	No. of Lactones	No. of moles of alkali consumed	
		Method A*	Method B†
I Ginkgolide A	3	2·1	2·4
II Ginkgolide B	3	1·9	2·5
III Ginkgolide C	3	1·9	2·8
IV Ginkgolide C O-methylether §	3	1·8	2·5
V Ginkgolide A O-methylether acid §	2	3·1‡	—
VI Bisnor-ginkgolide A	2	1·8	—

* Compound in 0·1 N aq. NaOH titrated with 1·0 N HCl.
† Solution of compound in 1·0 N aq. NaOH evaporated under reduced pressure at 50°C. Residue redissolved in water (to 0·1 N NaOH) and titrated with 1·0 N HCl.
‡ One mole of alkali consumed by the free carboxyl.
§ The OMe groupings do not correspond.

Compound IV in *Table 3* is a ginkgolide C methyl ether, in which the secondary hydroxyl group of the hydroxylactone moiety is methylated; compound V is a derivative having a cleaved ring C but will not be discussed in this article; compound VI is discussed later (paras III i and l).

Attempts to establish the number of lactone rings from the mass spectrum of the H_2O^{18}-treated compound

Treatment of ginkgolide A with methyl iodide and potassium carbonate in acetone yielded the dimethyl ether, which unlike other ginkgolide derivatives, gave a clear molecular ion peak at m/e 436 (see *Table 1*).

Since all the lactone groups are apparently cleaved under the conditions used in the potentiometric titrations (Method B, *Table 3*), it was thought that alkaline cleavage of the lactones, with 1N sodium hydroxide in water containing 30 per cent H_2O^{18} and under the same conditions as above, followed by recyclization on acidification would result in incorporation of O^{18} into all the lactone groups. Assuming free rotation about the C—C axis of the carboxylate groups, and hence a 50:50 chance of O^{18} incorporation, it should be possible to calculate the number of lactone groups in the molecule

from the ratios of the isotope peak-heights. Equations relating the intensities of the M + 2, M + 4, M + 6, . . . peaks and the number of lactone rings have been derived and are shown in *Figure 2*; (a) and (b) denote, respectively, the content of O^{18} and O^{16} (in the present case 0·30 and 0·70). In the calculated mass spectra shown in *Figure 2*, relative peak intensities derived from these equations are superimposed on the relative peak intensities arising from C^{13}.

Surprisingly, a comparison between the observed and calculated spectra seemed to indicate that only two and not three lactone functions were present. In view of the results of potentiometric titration, this anomaly should be interpreted by assuming an S_N2 type O-alkyl fission of one of the lactone rings, which is followed by another inversion at the hydroxyl-bearing carbon atom upon recyclization; the O^{18} atom thus is incorporated in the opened hydroxy-carboxylate form but is expelled again upon lactone formation. Such O-alkyl fission in one of the lactone rings is not unexpected if one considers the severely hindered cage structure of the ginkgolides.

(d) Reactions of rings F/A; nature of protons A–D

Reactions and spectroscopic data leading to the partial structure comprising rings F and A are summarized in *Figures 3 and 4* (for n.m.r. see para VI). The *tertiary* hydroxyl group of ginkgolide A was readily dehydrated by
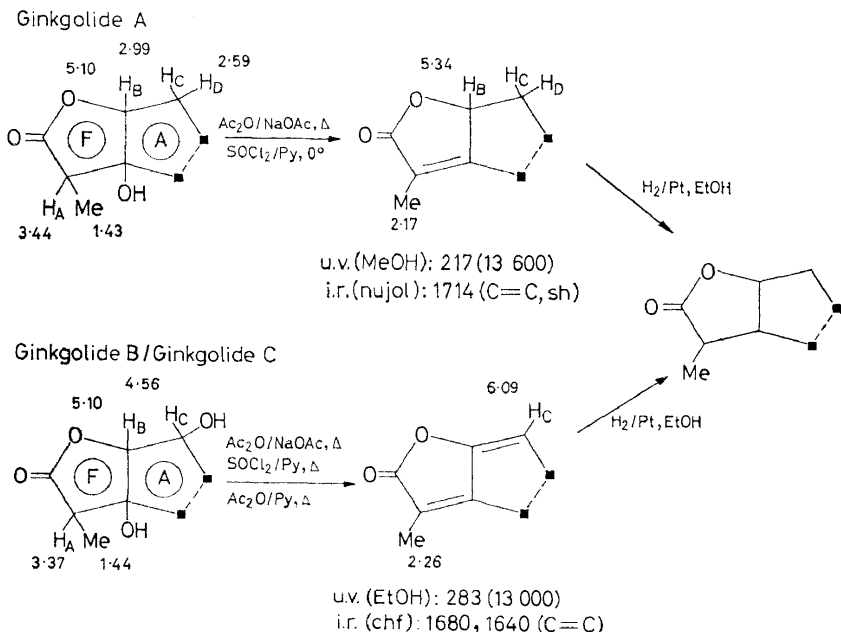

Figure 3. Reactions of rings F/A; nature of protons A–D. Small squares (■ .... ■) denote carbon atoms carrying no hydrogens as derived from n.m.r. data (measured in tetrafluoroacetic acid)

boiling in acetic anhydride–sodium acetate or treatment at $0°$ with thionyl chloride in pyridine, with concomitant conversion of a secondary methyl to an olefinic methyl. Furthermore, monoanhydro-ginkgolide A was readily hydrogenated to its dihydro derivative. The dehydration presumably proceeds through the acetate or thionyl ester of the tertiary hydroxyl function, since mild treatment of ginkgolide A diacetate with sodium acetate readily gave rise to monoanhydro ginkgolide A monoacetate.

Proton D is replaced by a hydroxyl function in ginkgolides B and C as indicated by n.m.r. data and formation of the dianhydro derivatives, the spectroscopic data of which are fully consistent with the $\alpha, \beta;\gamma,\delta$-unsaturated γ-lactone moiety, and with this the part structures shown are established.

The only difference between ginkgolides A and B is the presence of this additional hydroxyl group in the latter since hydrogenation of dianhydro-ginkgolide B and monoanhydro-ginkgolide A afforded the same product; ginkgolide C has one more hydroxyl group than ginkgolide B.

(e) Cleavage of rings F and A

When the dianhydro-ginkgolide C derivative, dianhydromonomethoxy-ginkgolide C monoacetate was hydrogenated in acetic acid with platinum, hydrogenolysis occurred to afford products having the partial structures shown in *Figure 4*.
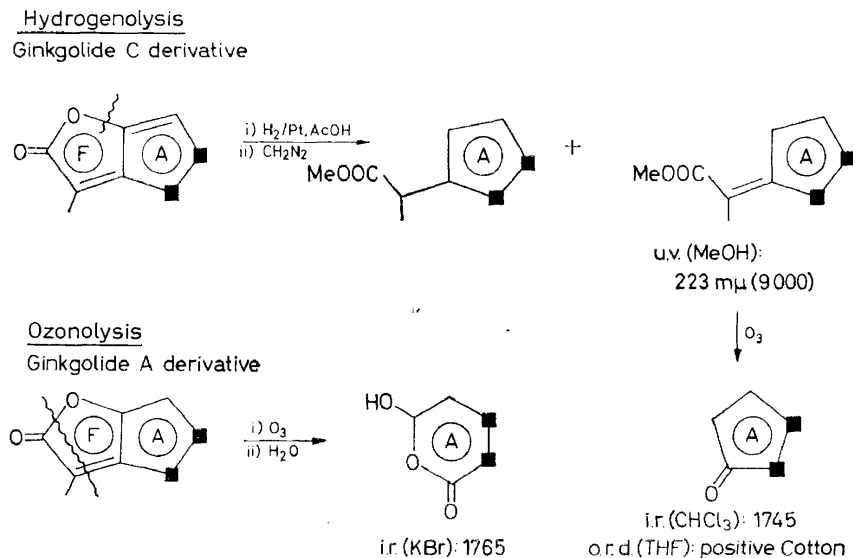
Hydrogenolysis
Ginkgolide C derivative

u.v. (MeOH):
223 mμ (9 000)

Ozonolysis
Ginkgolide A derivative

i.r. (KBr): 1765   i.r. (CHCl$_3$): 1745
o.r.d. (THF): positive Cotton

Figure 4. Cleavage of rings F and A under conditions of hydrogenolysis and ozonolysis

Ring A is five-membered because ozonolysis of the α,β-unsaturated ester gave a ketone with an i.r. absorption at 1745 cm^{-1} (CHCl$_3$). Ozonolysis of monoanhydro-ginkgolide A monoacetate resulted in a Baeyer–Villiger type

oxidation of the product to give the lactol shown. Both reactions provide further support for the structures of rings F and A.

(f) Protons E, F, G and H (Ring B)

The n.m.r. signals due to the moiety comprising the isolated four-proton system E–H in ginkgolide A and ginkgolide B could not be fully analysed in a straightforward manner because of the overlap of signals E–G; however, proton G is fortunately substituted by a hydroxyl in ginkgolide C and accordingly ginkgolide C and its derivatives were quite suited for n.m.r. (*Figure 5*). The spectra can only be satisfactorily explained by the arrange-
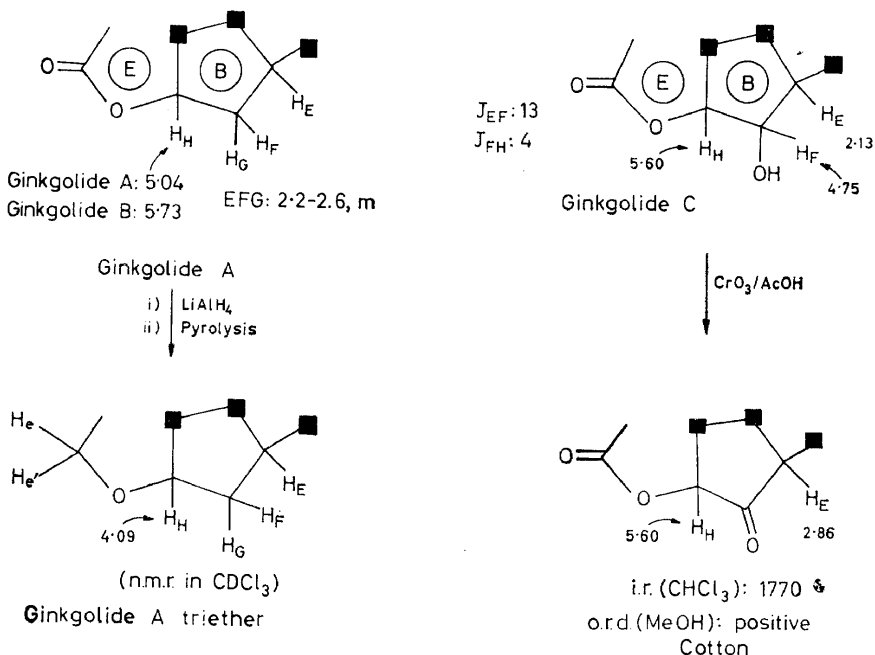
Figure 5. N.m.r. signals due to Protons E, F, G, and H (Ring B)

ment CH_H–CH_FOH–CH_E; moreover, chromic acid oxidation of ginkgolide C yielded a ketone, the formation of which was supported by the appearance of a positive Cotton effect in the o.r.d. curve. Furthermore, this ketone group absorbed at 1770 cm^{-1} in the i.r., and accordingly it is a five-membered ring ketone.

The lowfield position of the proton H signal (5·04–5·73 p.p.m.) in the four ginkgolides obviously requires this proton to be α to a strongly electron-attracting group. In the so-called ginkgolide A-triether (*Figures 6–8*), in which all lactonic carbonyl groups have been reduced to methylene groups, without any change in the ring system, the proton H signal coupled only to protons F and G and not to one of the newly introduced methylene protons. This indicates that in the original ginkgolides proton H is attached to a carbon atom linked to an oxygen, and cannot be α to the carbonyl end of a
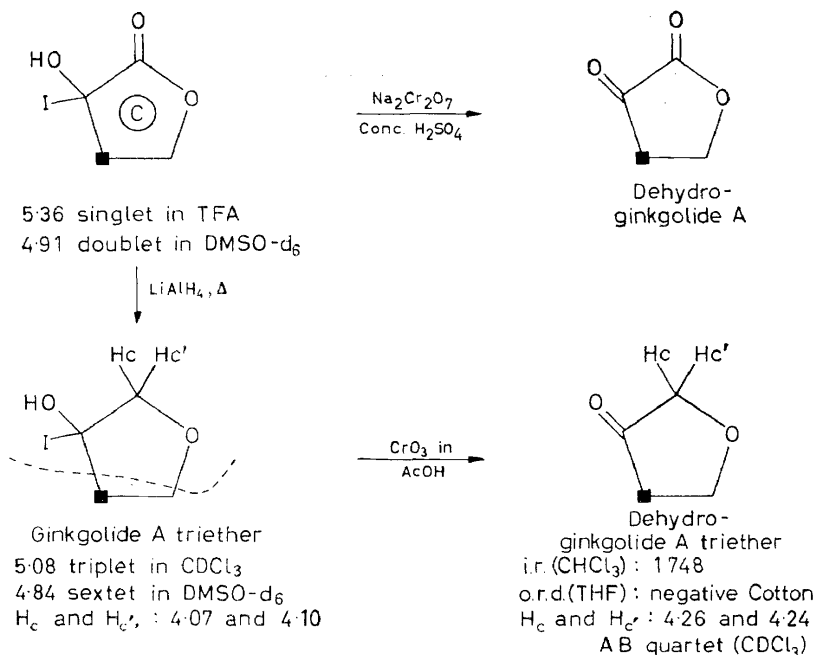
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Na$_2$Cr$_2$O$_7$
Conc. H$_2$SO$_4$

Dehydro-
ginkgolide A

5·36 singlet in TFA
4·91 doublet in DMSO-d$_6$

LiAlH$_4$, Δ

Hc Hc'

HO
I

Hc Hc'

CrO$_3$ in
AcOH

Ginkgolide A triether
5·08 triplet in CDCl$_3$
4·84 sextet in DMSO-d$_6$
H$_c$ and H$_{c'}$, : 4·07 and 4·10

Dehydro-
ginkgolide A triether
i.r.(CHCl$_3$) : 1748
o.r.d.(THF) : negative Cotton
H$_c$ and H$_{c'}$: 4·26 and 4·24
A B quartet (CDCl$_3$)

Figure 6. Proton I and the hydroxylactone (Ring C)

lactone. Moreover, in the triether, the chemical shift of proton H is shifted upfield to the extent of 0·6 p.p.m. (*i.e.*, 4·09 p.p.m. in CDCl$_3$; proton H in ginkgolide A dimethyl ether, a ginkgolide A derivative which is soluble in CDCl$_3$, appears at 4·64 p.p.m.). Therefore, the above mentioned oxygen atom should be further linked to a carbonyl group, so that proton H is α to the oxygen end of a lactone as shown in *Figures 6 and 7*.

250   350   450 mμ
-1500

o.r.d.

-100
u.v.
1000

$[\phi] \times 10^{-2}$
-200
500

×10

-300
0
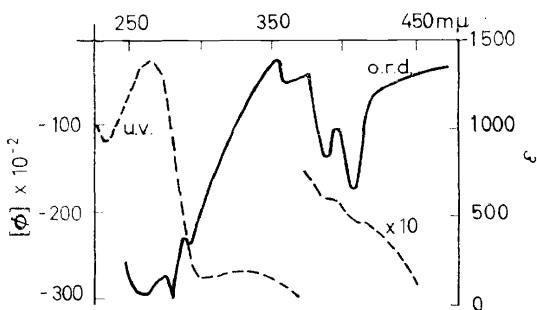
Figure 7. Optical rotatory dispersion and ultraviolet curves of dehydro-ginkgolide A

(g) Proton I and the hydroxylactone (Ring C)

Oxidation of ginkgolide A with a reagent stronger than the usual chromic acid washing solution gave dehydro-ginkgolide A lacking the proton I; these were the only conditions under which ginkgolide A could be successfully

oxidized, but the yield was almost quantitative. The u.v. and o.r.d. curves clearly indicated that an α-dicarbonyl function had been formed; thus ginkgolide A contains an α-hydroxylactone group which is oxidized to a keto-lactone. The presence of the hydroxylactone group received further support from the fact that (i) the n.m.r. of ginkgolide A-triether (*Figures 6 and 11*) showed a triplet (proton I) at 5·08 p.p.m. in CDCl₃, which became a sextet in DMSO-d₆; (ii) a clear M-60 peak was observed in the mass spectrum. Finally, the hydroxylactone is considered to be five-membered because oxidation of the triether gave a ketone (dehydro-ginkgolide A triether, see *Figure 6*) with an i.r. band at 1748 cm⁻¹.

(h) The three lactones

Partial structures incorporating lactones F, E, and C, have been derived in the foregoing paragraphs, but since the points of attachment of the carbonyl group in lactone E and the oxygen in lactone C have not been defined these two lactone groupings could be one and the same. This point can be clarified from the following observation. When ginkgolide A was heated for 30 min at 160° in 50 per cent sodium hydroxide and then acidified, a product designated bisnor-ginkgolide A, because of the loss of two carbon atoms ($C_{18}H_{24}O_7$, from mass spectrum), was obtained in *ca.* 50 per cent yield. Bisnor-ginkgolide A has only *two* lactone rings (by titration, *Table 3*), and it was clear that lactones F and E had not undergone any change since the n.m.r. signals arising from protons A, B–D, and H–E remained essentially the same (excepting that proton E is further split by *ca.* 9 c/s, see *Figure 9*). Instead of the hydroxylactone function, bisnor-ginkgolide A contains a *hemiacetal* as evidenced by its oxidation to a trilactone. Convincing evidence that it is the hydroxylactone C which has been lost is provided by the detection of oxalic acid in the alkali reaction mixture, *i.e.*, the two carbon
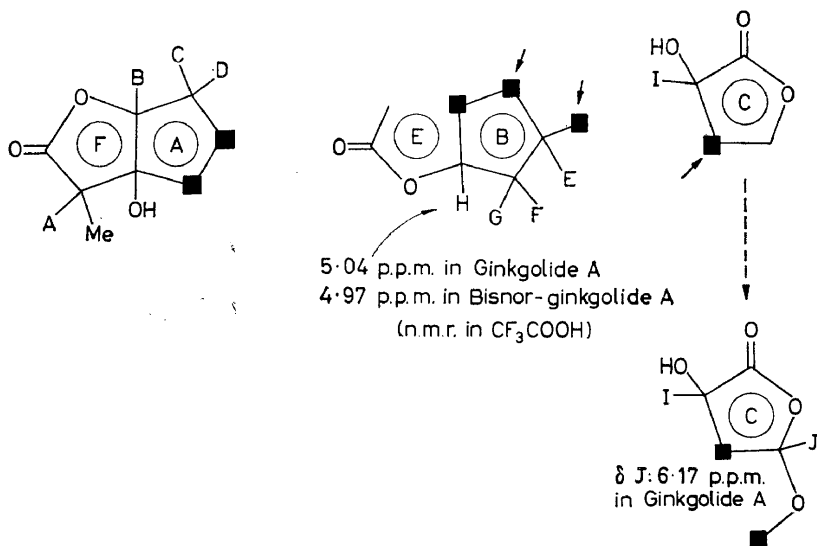

Figure 8. Nature of the three lactones

atoms lost in ginkgolide A originate from the hydroxylactone group, which therefore *cannot* be identical with lactone E. The three lactone groups C, E, and F are therefore as shown in *Figure 8* and account for eight of the nine oxygen atoms in ginkgolide A.

As already mentioned (in para III b) the n.m.r. spectra of the ginkgolides exhibit a conspicuous *singlet* J at the lowfield of *ca*. 6·2 p.p.m., besides that of the other lowfield proton I on ring C. The appearance of the proton J signal at such lowfield can only be accounted for by its presence in one of the partial structures –O–CH$_J$–CO–O– or –O–CH$_J$–O–. Since the n.m.r. spectrum of the triether (*Figures 6 and 11*) establishes that in ginkgolide A *one* of the lactone carbonyl groups has *no* α-proton and that protons A and I are α to the other two carbonyls, respectively, it follows that proton J must be located between the ether oxygen and the oxygen of lactone C, viz., –C(OH)(H$_I$)–CO–O–C(H$_J$)–O–C–.

(i) The formation of bis-norginkgolide A

As mentioned in *Figure 8*, vigorous alkali treatment of ginkgolide A afforded bisnor-ginkgolide A, resulting from the loss of a two carbon chain presumably in the form of glyoxylic acid (which yields the oxalic acid mentioned previously via a Cannizzaro reaction). The n.m.r. spectrum indicated that the only changes involved were related to ring C, and that rings A, B, E, and F remained intact. A hemiacetal function is present in bisnor-ginkgolide A since: (i) the presence of a carbinyl proton J is indicated by the lowfield signal at 5·63 p.p.m., which forms part of an AB type quartet
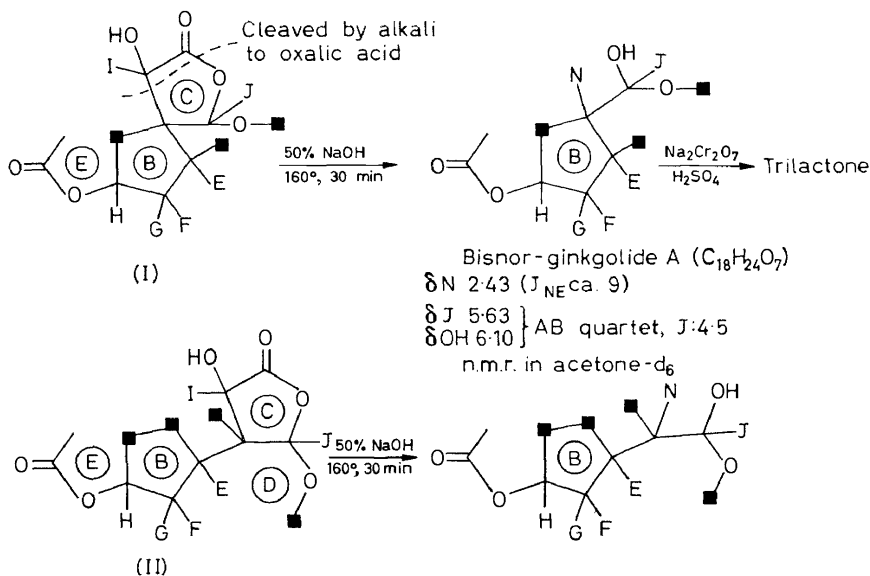

Figure 9. The formation of bis-norginkgolide A

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with a hydroxyl signal, in acetone-d_6; and (ii) oxidation gave a trilactone consisting of lactones E, F, and the new lactone derived from the hemiacetal.

The bisnor compound was extremely informative in that it was the only derivative that had a proton (N) strongly coupled to the hitherto isolated proton system E-H through proton E, the coupling constant J_{NE} being *ca.* 9 c/s. Undoubtedly, this new proton N should be placed at the blocked carbon in ring C (see short straight arrows in *Figure 8*) after loss of the two-carbon fragment, and the large N-E coupling further requires protons N and E to be on adjacent carbon atoms, *i.e., ring C should be attached to one of the blocked carbons (marked with arrows) adjacent to proton E,* and this leads to the two possibilities (I) and (II) depicted in *Figure 9.* In further confirmation of these two partial structures, proton N is slightly coupled to proton J.

(j) The possible structures

We are now left with the problem of linking rings F/A and the *tert*-butyl group with either part structure (I) or (II). Only four pairs of structures (*Figure 10*) can be constructed, each pair differing only in the mode of attachment of rings F and A (cf., Ia and Ib). Of these four pairs, *only two* (pairs I and II) are in accord with the minimum requirement for a facile photochemical rearrangement (cf. para III m), namely close proximity of the *tert*-butyl group and the hydroxyl function of ring C.
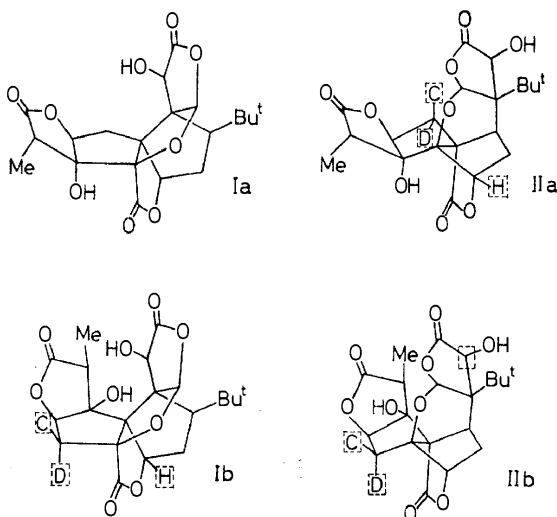

Figure 10. The possible structures

The structures (Ib) and (IIa) are eliminated on the following grounds. The proton H signal is at 5·04 p.p.m. in ginkgolide A, but this is subject to considerable deshielding when proton C or D is replaced by a hydroxyl group, *i.e.,* it appears at 5·73 p.p.m. in ginkgolide B, 5·60 p.p.m. in ginkgolide C, and 5·49 p.p.m. in ginkgolide M. The protons in question are too

widely separated in structures (Ib) and (IIa) to account for this spatial interaction.

Structure (IIb) is also eliminated by comparing the chemical shifts of protons C and D in ginkgolide A-triether (all carbonyl groups reduced to methylenes, no skeletal change) and dehydro-ginkgolide A-triether (secondary hydroxyl in the triether oxidized to a carbonyl, see *Figure 6*). Thus in the latter compound, protons C and D have undergone an upfield shift to the extent of 0·34 and 0·61 p.p.m., respectively, but the structure (IIb) cannot account for this large anisotropic effect of the carbonyl group.

We are now left with only one structure, (Ia), which is fully substantiated by all experimental data, both chemical and spectroscopic, some of which are discussed below. For the sake of simplicity, structures in the following figures depict the relative and absolute stereochemistry as well.

(k) Ginkgolide A-triether and the deuterotriether

Lithium aluminium hydride reduction of ginkgolide A in dioxane, acid decomposition of the complex, and heating of the resulting polyol at 150°/5 mmHg for 2–3 hours, afforded a key derivative which we named ginkgolide A-triether (*Figures 5 and 6*). The M^+ peak indicated that in terms of molecular weight, the only change was the conversion of all three lactonic carbonyl groups into methylene groups, and in fact, full analysis of its n.m.r. spectrum and comparison with the ginkgolide A spectrum clearly showed that no skeletal rearrangement was induced by this reaction sequence.

This "triether" was of great importance since it permitted characterization of the moieties connected to the lactone groups. The n.m.r. spectra of the ginkgolides are too simple because they consist of several unrelated (*i.e.*, uncoupled) proton-systems A–D, E–H, I and J, isolated by fully substituted carbon atoms or lactone rings. In the triether, however, the too-simple n.m.r. spectrum of ginkgolide A is converted into a more complex spectrum, thus enabling one to derive more information on the arrangements of protons. Although the ginkgolide A triether spectrum was quite complex, the entire region was analysed in detail by taking full advantage of solvent shifts to reveal overlapping signals, and using the techniques of double and triple resonance. The spectrum measured in deutero-chloroform after addition of heavy water is shown in *Figure 11*; the more simple methyl resonance region is omitted. The protons introduced by the lithium aluminium hydride reduction step are indicated in lower case lettering in the *Figure*. All chemical shifts and coupling constants shown in the *Figure* were deduced by first-order analysis, and several small long-range couplings were also disclosed.

After full analysis of this complex spectrum the n.m.r. of the deutero-triether, in which all protons expressed in lower case lettering have been replaced by deuterium, was measured and it was gratifying to find the n.m.r. assignments shown in the upper trace to be fully confirmed. A comparison of the two traces shows that all signals and large couplings involving protons indicated in lower case lettering have disappeared in the deutero derivative. Moreover, the close similarity between the spectrum of the deutero

compound and that of ginkgolide A (*Figure 15*) should be noted. This is of course not unexpected if one considers that the difference between the two compounds, resides only in the replacement of the three CO groups with CD₂ groups. Thus the overall picture in the ginkgolide A to triether to
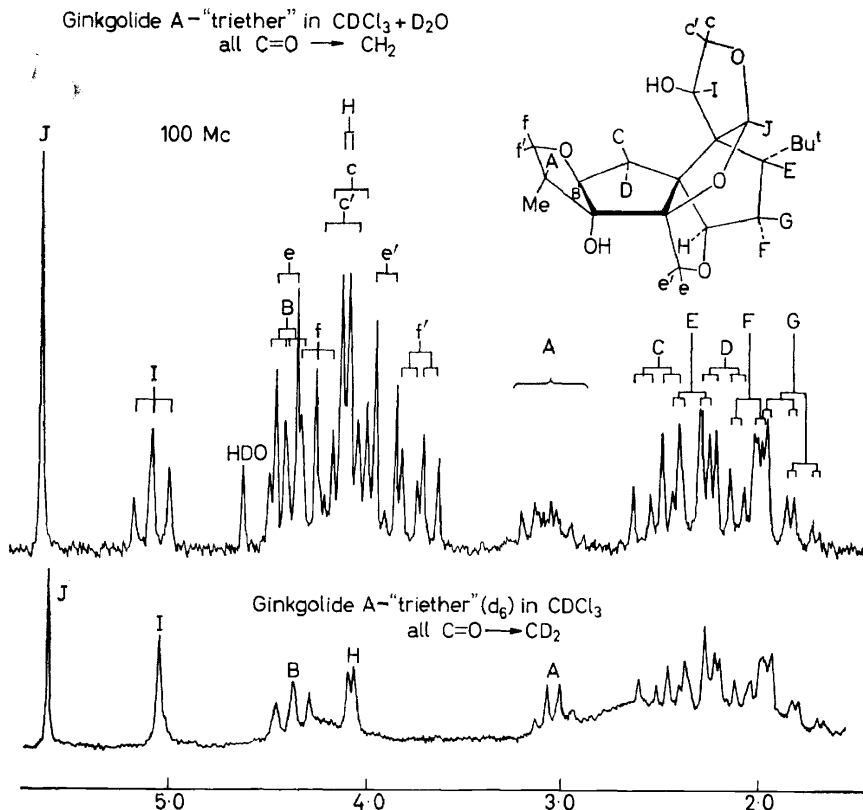

Figure 11. N.m.r. spectra of Ginkgolide A-triether and the deuterotriether

deuterotriether conversions is, complication of the spectrum by changing CO to CH_2, and resimplification by changing CH_2 to CD_2.

Finally, the spectrum of the ketone shown in *Figure 6, i.e.,* the dehydrotriether, was also fully analysed, and a comparison with the original triether spectrum revealed significant shifts, especially in signals C and D (cf. para III j), resulting from the anisotropy of the newly introduced ketone group. All other significant shifts were fully consistent with the derived structure.

(l) Bisnor-ginkgolide A

Bisnor-ginkgolide A and its dehydro derivative, also played important roles in clarifying the surroundings of the lactone groups (*Figures 8 and 9*),

THE GINKGOLIDES

and can be represented as shown in *Figure 12*. Removal of the two-carbon chain in the form of glyoxylic acid (detected as oxalic acid) is presumably due to alkaline cleavage of the 1,3-diol formed upon opening of lactone ring C.
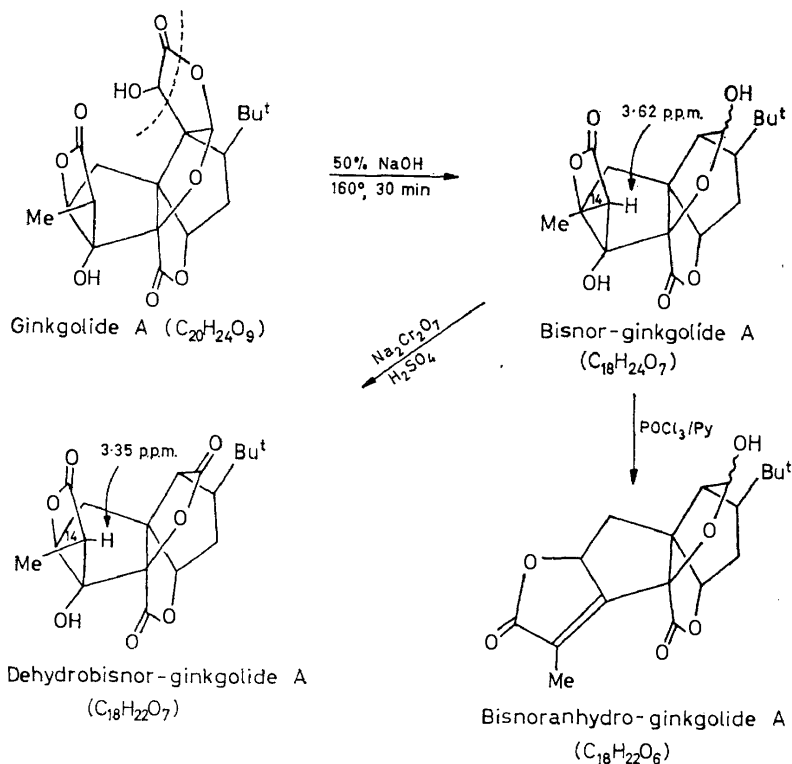

Ginkgolide A ($C_{20}H_{24}O_9$)

50% NaOH
160°, 30 min

Bisnor-ginkgolide A
($C_{18}H_{24}O_7$)

$Na_2Cr_2O_7$
H_2SO_4

Dehydrobisnor-ginkgolide A
($C_{18}H_{22}O_7$)

POCl$_3$/Py

Bisnoranhydro-ginkgolide A
($C_{18}H_{22}O_6$)

Figure 12. Reactions of bisnor-ginkgolide A and related compounds

(m) Photodehydroginkgolide A

It was mentioned in *Figure 6* that drastic oxidation of ginkgolide A afforded dehydro-ginkgolide A containing the α-ketolactone ring. However, examination of the properties of this straightforward oxidation product was complicated by the ready occurrence of an unsuspected photochemical rearrangement.

For a long time, the secondary hydroxyl was considered to form part of a hemiacetal group since the o.r.d. curve of the oxidation product was thought to exhibit no Cotton effect above 250 mμ, a behaviour in accord with a "tetralactone" structure. However, it was later discovered that the o.r.d. curve measured actually belonged to the photodehydro-ginkgolide A which had been formed during storage of the dehydro-ginkgolide A (*Figure 13*). Only after measuring the n.m.r. spectrum of a sample that had been kept for some time did it become apparent that the dehydro-ginkgolide A had been

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P.A.C.—8

completely converted into photodehydro-ginkgolide A and that the *tert*-butyl group had surprisingly been converted into a gem-dimethyl group and a methylene group.
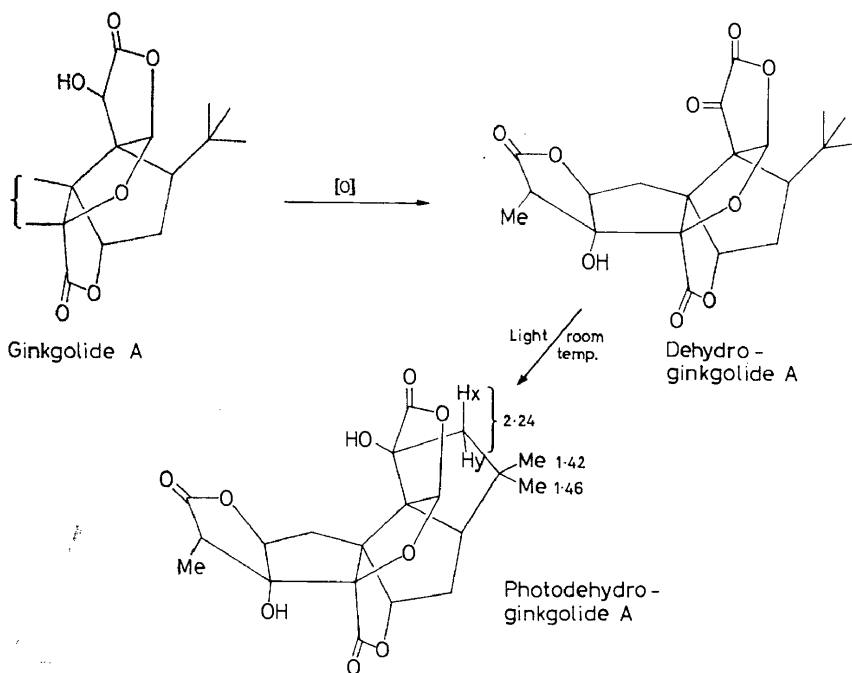

Figure 13. Formation of Photodehydroginkgolide A

This suggested that a photochemical reaction had taken place. The u.v., o.r.d. (*Figure 7*) and n.m.r. spectroscopic data on the fresh oxidation product indeed revealed the presence of a keto-lactone system and the intact *tert*-butyl group. The dehydro-ginkgolide A was rapidly transformed into the photo-rearrangement product, photodehydro-ginkgolide A, by either leaving a solution or a spot on a thin-layer chromatographic plate in the light; in the latter case the conversion was complete after 4 hours. Apparently, this exceptionally facile photo-cyclization is caused by the long-wavelength u.v. absorption band (*Figure 7*).

A model of ginkgolide A indicates that the spatial disposition of the *tert*-butyl group (α-configuration) and the carbonyl group is ideally suited for hydrogen abstraction from one of the *tert*-butyl methyl groups to give photodehydro-ginkgolide A, which now has a unique cage structure built up of *seven* strain-free five-membered rings.

IV. STEREOCHEMISTRY OF THE GINKGOLIDES

The configurations at seven asymmetric centres, C–1, C–2, C–3, C–7, C–8, C–10, and C–14 have to be considered for ginkgolide C, which contains the largest number of asymmetric carbon atoms.

The *absolute stereochemistry* of the ginkgolides can be simply deduced as follows. The facile photochemical rearrangement of dehydro-ginkgolide C requires the *tert*-butyl group and the C_9–C_{10} bond to be situated on the *same side* of ring B. Since monomethoxyoxo-ginkgolide C (OMe at C–10 and ketone at C–7) exhibits a positive Cotton effect in its o.r.d. curve, it follows from the octant diagram (*Figure 14*) that the ginkgolides must have the absolute stereochemistry depicted in *Figure 14*. The remaining configurations were established as follows.

Ginkgolide C   Isoginkgolide C triacetate   Octant diagram of monomethoxyoxo - ginkgolide C (positive Cotton effect)

Monoanhydro - ginkgolide C derivative   (1)   Dihydro derivative   (2)   Epimerized dihydro derivative   (3)
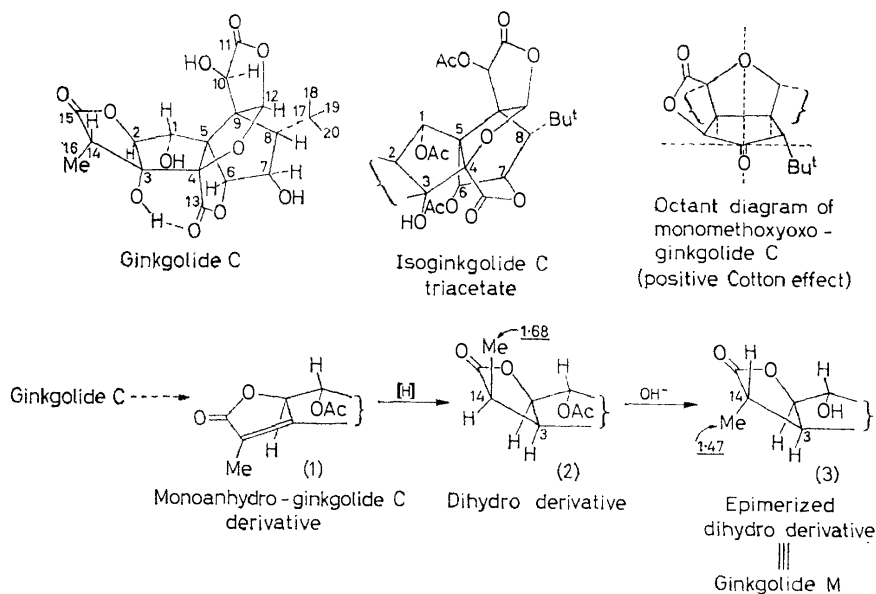
Ginkgolide M

Figure 14. Stereochemistry of the ginkgolides

(*i*) *C–10*: Proton I must be on the same side of ring C as the *tert*-butyl since these groupings participate in an intramolecular Overhauser effect (see Table under *Figure 15*).

(*ii*) *C–7*: When ginkgolide C is acetylated by boiling in Ac_2O–NaOAc, an isotriacetate is readily formed which is reconverted to ginkgolide C itself upon saponification with MeOH–NaOMe. The isotriacetate can be further acetylated to isoginkgolide C tetraacetate. These acetates are isomeric to the normal tri- and tetraacetates, and are formed by translactonization of lactone E (from C–6 to C–7, see *Figure 15*). This requires an S-configuration at C–7 which is in line with the coupling constants J_{EF} 13 c/s and J_{FH} 4 c/s in ginkgolide C, and J_{EF} 0 c/s and J_{FH} 4 c/s in the isomeric acetates. The detected nuclear Overhauser effects in the normal and isomeric acetates is also consistent with the structures shown.

(*iii*) *Rings F/A*: Cleavage of ring F with alkali and acidification readily gives back the starting material, thereby indicating that the γ-lactone is *cis*-fused.

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(*iv*) *C–14 and C–3*: A *trans* arrangement of 14-H and 3-OH is suggested by the ready formation of monoanhydro-ginkgolide C under mild conditions (*Figure 3*).

This is established by the following evidence which is described in only very brief form. A monoanhydro-ginkgolide A derivative *1* was hydrogenated to the corresponding dihydro derivative *2*, which upon mild base treatment was epimerized at C–14 to the dihydro derivative *3*, which was identical in every respect with ginkgolide M. The C–14 epimerization was evident from the appearance of the methyl signals of *2* and *3*, at 1·68 and 1·47 p.p.m., respectively. Since hydrogenation must have occurred from the same side of ring F in compound *1*, the first dihydro derivative *2* has the 14–H and 3–H in a *cis* relation, while the epimerized dihydro derivative, which is ginkgolide M, has them in a *trans arrangement* and is also the thermodynamically more stable. Furthermore, the ring F/A configurations in all ginkgolides are identical as indicated by comparisons of the chemical shifts of protons H_B, H_C and J_{BC}, and the fact that mild base treatment of the natural products induces no epimerization of the 14–Me group. Namely, the 14-methyl group adopts the thermodynamically more stable configuration.

(*v*) *C–3 and C–2*: The 3-OH and 2-H are α,α-*cis*. This conclusion is based on the fact that the chemical shift of 14-H, which as deduced above is *trans* to the 3-OH, is considerably affected by the oxygen function at C-12 and hence should be β. Namely, it is at 3·62 p.p.m. in bisnor-ginkgolide A (*Figure 12*) but at 3·35 p.p.m. in dehydrobisnor-ginkgolide A, the 0·27 p.p.m. diamagnetic shift being in line with the spatial arrangement of the C–12 oxygen function and a 14-βH.

The ready methylation of the 3-OH in ginkgolide A upon treatment with MeI/K_2CO_3 in acetone can also be understood on the basis of an intramolecular H-bonding between the 3-OH and 13-CO (i.r. evidence), which would serve to weaken the O–H bond and facilitate methylation.

(*vi*) *C–1*: The 1-OH in ginkgolides B, C, and M adopts an α-configuration because the 6-H is subject to considerable deshielding by introduction of the additional OH. Molecular models show that a 1α-OH is in the sample plane as that of 6-H. The 6-H chemical shifts are as follows: ginkgolide A, 5·04; ginkgolide B, 5·73; ginkgolide C, 5·60; ginkgolide M, 5·49 p.p.m.
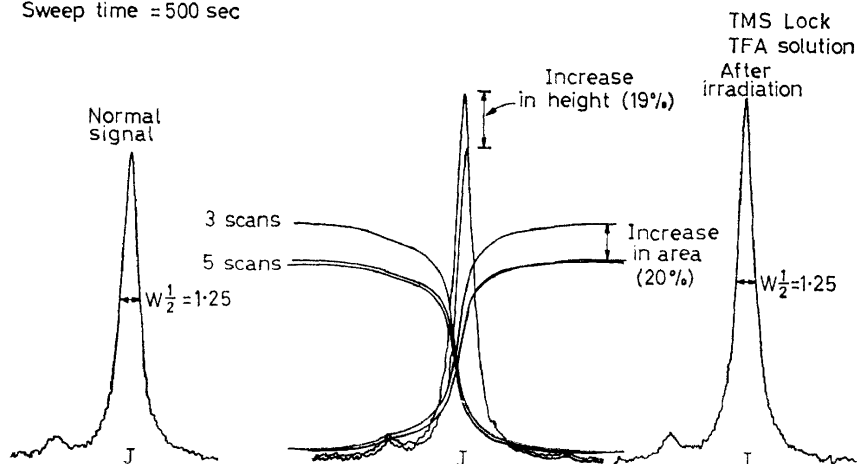

V. THE NUCLEAR OVERHAUSER EFFECT (NOE)

In an attempt to relate the isolated proton systems, by using n.m.d.r. (double resonance) techniques to search for some indication of small long range couplings between protons belonging to different systems, it was found that irradiation (saturation) of the *tert*-butyl group caused a significant increase (*ca.* 30 per cent) in the height of the I and J proton signals, but did not cause any great decrease in the half-band width of these signals. Moreover, in the case of ginkgolide C, the heights of signals due to protons E and F were also found to increase on irradiation of the *tert*-butyl group. Since it seemed unlikely that all four of these protons could be coupled to the *tert*-butyl by a through-bond mechanism, the possibility of through-space coupling or an effect involving a relaxation mechanism was considered.

Proton J at 6·26 p.p.m.

NUCLEAR OVERHAUSER EFFECTS*

Proton observed	Proton irradiated	Ginkgolide A	Ginkgolide B	Ginkgolide C	Ginkgolide C tetraacetate	Isoginkgolide C tetraacetate	Isoginkgolide C triacetate	Ginkgolide A di OMe, mono CO₂Me
I	t-Butyl	30 [32]	25 [24]	20 [24]	33 [36]	27 [37]	30 [32]	Nil [<4]
J	,,	22 [22]	21 [23]	20 [19]	22 [21]	Nil	Nil	22 [28]
F	,,	-	-	4 [10]	16 [13]	19 [22]	24 [23]	-
E	,,	-	-	-	6 [16]	13 [17]	14 [14]	-
H	,,	Nil	Nil	Nil	Nil	6 [10]	12 [15]	Nil
J	E	-	-	Nil	Nil	10 [17]	7 [21]	-
E	J	-	-	Nil	Nil	23 [30]	20 [27]	-

* Given as the percent increase in integrated intensity on irradiation; the figure in parenthesis is the percent increase in height of the signal. Solutions were in trifluoroacetic acid; signals recorded at 50 c/s/500 sec sweeps and integrated at 50 c/s/100 sec sweeps, on a Varian HA-100.

Figure 15. The nuclear Overhauser effect: Effect of irradiating the *tert*-butyl at 1·30 p.p.m. ginkgolide C

In a series of recent communications[15], it has been shown that in certain rigid molecules, it is possible to observe an intramolecular Overhauser effect when a large contribution to the relaxation of a particular proton comes from one or more closely situated protons in the same molecule. In such a case, saturation (*e.g.*, by double resonance) of the proton(s) responsible for the relaxation of the proton in question causes an appreciable increase (up to

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50 per cent) in the integrated intensity of the signal due to the latter proton[15].

The results of chemical and spectroscopic studies carried out indicate that the ginkgolides possessed a rigid cage-like skeleton, and it therefore appeared very likely that the increase in the height of the signals from the I, J, E, and F protons in ginkgolide C, on irradiation of the *tert*-butyl group, might well arise from a nuclear Overhauser effect.

Integration of the I, J, E, and F proton signals both before and during irradiation of the *tert*-butyl group showed that a marked increase in the integrated intensities of these signals resulted from saturation of the *tert*-butyl group. Some results are listed in the *Table* (under *Figure 15*) and an example is shown in *Figure 15*. On the other hand, irradiation of proton J, for example, did not cause any appreciable increase in the *tert*-butyl signal. In the case of isoginkgolide-triacetate and -tetraacetate (see *Figures 14 and 15*), isomers of the normal acetates, it is of interest to note that proton J does *not*
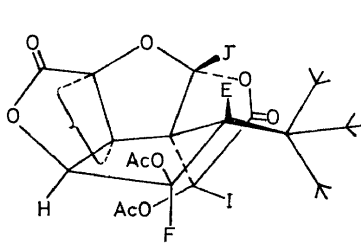

Ginkgolide C tetraacetate
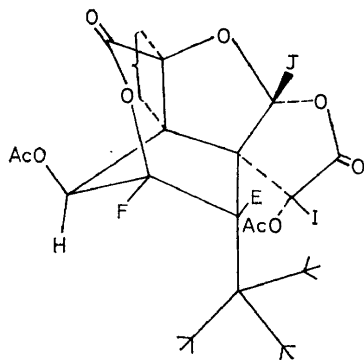
Isoginkgolide C tetraacetate

exhibit any nuclear Overhauser effect but instead the *tert*-butyl protons now appear to be largely responsible for the relaxation of proton H which is not so affected in the case of the other compounds in the *Table*. It was significant that the nuclear Overhauser effect was not exhibited any more by proton I in the ginkgolide A derivative shown in the last column of the *Table* (in this derivative, lactone C is opened and the resulting three OH groups at 10, 12 and 13 are methylated). The importance of these results in assigning structures and stereochemistry to the ginkgolides is obvious, since in order for this relaxation mechanism to operate the protons in question must be very closely situated[15].

VI. N.M.R. SPECTRA OF GINKGOLIDES A, B and C

The relatively simple n.m.r. spectra of these complicated compounds (*Figures 16–18*) need little comment and will not be discussed further. However, it may be commented that due to the rigid and extensive cage
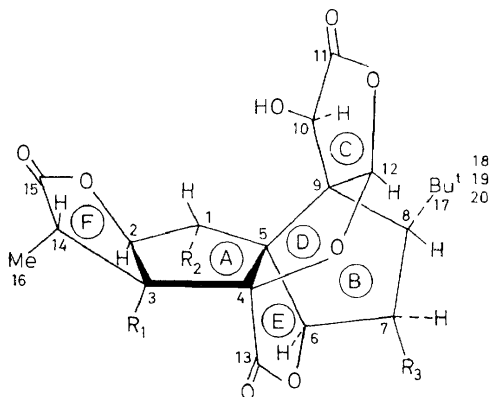
Figure 16
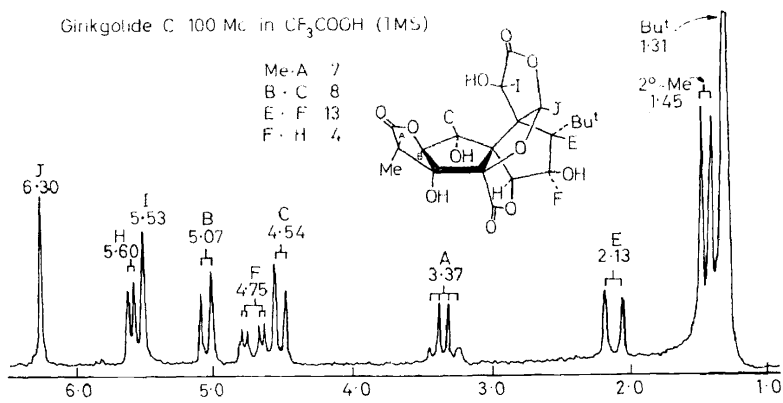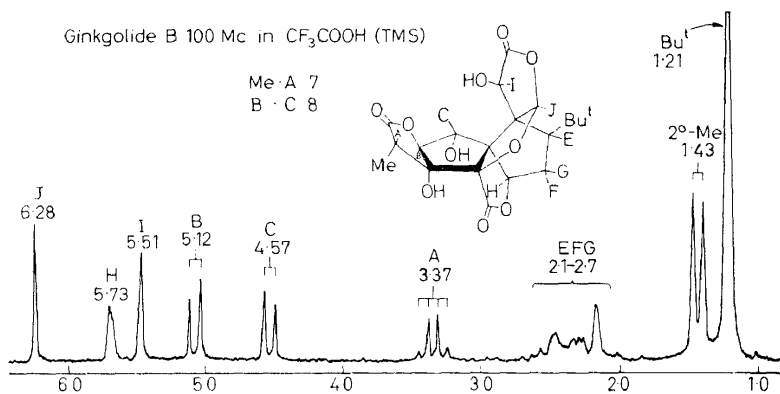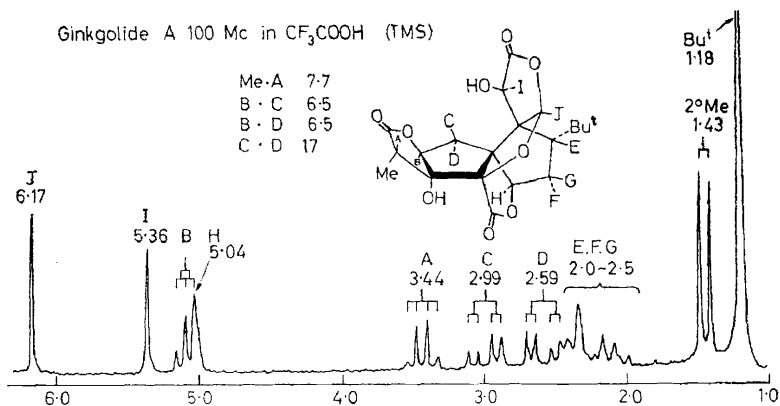
Figure 17

Figure 18

Figures 16–18. N.m.r. spectra of ginkgolides A, B and C

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structure, it should not be surprising if the presence of bond-angle distortions and bond anisotropy effects in these molecules lead to rather unusual chemical shifts and coupling constants in their n.m.r. spectra. For this reason, in most of the described n.m.r. analyses, only the differences in chemical shifts between two or more derivatives were considered. Moreover, the familiar relation between dihedral angle and coupling constant has not been unduly employed.

VIII. FULL STRUCTURE OF THE FOUR GINKGOLIDES

The ginkgolide structure is built up very comfortably from six five-membered rings (*Figure 19*), and the chemical inertness of the ginkgolides and their rigid cage structures are not unrelated.

	R₁	R₂	R₃
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide M	H	OH	OH

Figure 19. Full structure of the four ginkgolides

Note added in proof. Some aspects of the work reported in this article have since been published.[16-20]

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