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THE GINKGOLIDES. I.

ISOLATION AND CHARACTERIZATION OF THE VARIOUS GROUPS

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The isolation of bitter substances from the leaves of Ginkgo biloba L. (Japanese: ICHO), a tree which is the sole living representative of the once widely distributed order Ginkgoales, was first reported by Furukawa in 1932 1). More recently, Sawada has reported 2) the results of further studies on these compounds to which he assigned C_{11} formulae on the basis of microanalysis only.

Studies on the root bark of the ginkgo tree were commenced in this laboratory in 1960 by the late Professor S. Fujise, who succeeded in isolating several crystalline bitter substances which proved to be extremely difficult to purify, due in part to their tendency to exhibit polymorphism. As described below, careful separation and purification of the mixture of bitter compounds afforded four distinct ginkgolides all of which are C_{20} compounds.

Further work, described in the following series of papers 3-6), has shown the ginkgolides to be unique cage molecules incorporating a tert-Bu group and six five-membered rings, the carbon skeleton being a spiro[4, 4]nonane system.

Extraction. A methanol extract of the undried chopped root bark (100 Kg) was concentrated to a syrup and then washed with benzene. The aqueous layer gave a solid which was crystallized from ethanol to give 50 g of a crude mixture of the ginkgolides. A solution of these crystals in acetone was absorbed onto celite, the celite was placed as a layer on top of a silica gel column, and the column was eluted with chloroform containing a trace of ethanol. This afforded a mixture of ginkgolide A (abbreviated to GA) and ginkgolide B (GB), a small amount of ginkgolide M (GM) and finally ginkgolide C (GC). The separation of GA and GB was extremely tedious and moreover was complicated by the tendency of GA to exhibit polymorphism.

Satisfactory results were achieved only after a 10-15 step fractional recrystallization procedure, the purity being checked either by NMR or optical rotation. The following yields were obtained: GA 10 g, GB 10 g, GC 20 g, GM 200 mg.

All the ginkgolides are bitter, have no melting point (dec. above 280°) and are extremely stable towards concentrated mineral acids; evaporation to dryness of a concentrated nitric acid solution of the ginkgolides results in recovery of crystalline starting material.

The molecular formulae were established by injection into the direct inlet system of a high resolution mass spectrometer; the relatively volatile GA dimethyl ether, $C_{22}H_{28}O_9$, showed a molecular peak at 436.168, (calcd., 436.173). As summarized in the following table the ginkgolides are $C_{20}H_{24}$ compounds differing in the number of oxygen atoms[9].

GA	$C_{20}H_{24}O_9$	$[\alpha]_D$ - 39°	(c 1.0, diox.)
GB	$C_{20}H_{24}O_{10}$	$[\alpha]_D$ - 63°	"
GC	$C_{20}H_{24}O_{11}$	$[\alpha]_D$ - 19°	"
GM	$C_{20}H_{24}O_{10}$	$[\alpha]_D$ - 39°	"

tert-Butyl group. The NMR[10] of all derivatives, excepting photodehydro-GA[4,5] showed a 9-proton singlet in the range 1.2-1.3 ppm. 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 seems to have been recorded for natural products. Moreover, the mass spectra have a *strong* peak (rel. intensity 50-100% of base peak) at 57.074, which corresponds to the stable tert-butyl cation, 57.070. The presence of the tert-butyl group was finally established by oxidation of GC under Kuhn-Roth conditions and isolation of pivalic acid, characterized as the crystalline p-bromophenacyl ester.

The hydroxyl groups. The number and nature of the hydroxyl groups were deduced by the conventional NMR techniques of comparing the spectra of the original ginkgolides with those of the acetates and by measurements of spectra in DMSO-d_6 and addition of D_2O or deuterated acid.

	sec-OH	tert-OH
GA	one	one
GB	two	one
GC	three	one
GM	three	-

Carbonyl functions. The IR spectra (KBr, MeCN, dioxane) of the ginkgolides show a strong but ill-defined absorption around 1780 cm^{-1}, which is apparently due only to lactone carbonyls since this band was replaced by carboxylate absorptions when, for example, an aqueous sodium hydroxide solution of GB 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 a KBr disk; GB was recovered upon dissolution of the KBr disk and acidification, thus indicating no chemical change apart from cleavage and recyclization of the lactone rings. The ease with which the ginkgolides undergo cleavage and relactonization means that all lactones must be essentially strain-free.

The presence of three lactone groupings was suggested by lactone titration under the following conditions. When a solution of the compound in 1. 0 N aqueous NaOH was evaporated under reduced pressure at 50°, and the residue (IR showed that all lactone groups had been cleaved) was redissolved in water and titrated with 1. 0 N HCl, GA, GB and GC consumed 2. 4, 2. 5 and 2. 8 moles, respectively of alkali.

The absence of a ketone grouping is also suggested by the plain negative RD curves exhibiting no Cotton effect in the range 250-700 mμ. The presence of three lactones and the absence of any other carbonyl grouping was established through the detailed NMR analysis 6) of "GA triether" (actually a tetraether) which showed that all three carbonyl groups had been reduced to methylene groups which are bonded to ether oxygens.

Establishment of the number of hydroxyl and lactone groups leaves one more oxygen function to be accounted for, and this is assigned to an ether linkage 3).

The ginkgolides contain no C=C double bonds as evidenced by the lack of olefinic peaks in their NMR spectra, the absence of any absorption near 1650 cm^{-1}, and the low end-absorption in the UV spectra (i. e., no double bond) of "GA triether" 6), ε at 204 mμ is 80. The ginkgolides are thus hexacyclic compounds.

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8) The final structures of the ginkgolides were presented at the 10th Symposium on the Chemistry of Natural Products, Tokyo, 1966.

9) All elemental analyses were consistent with molecular formulae.

10) Owing to solubility problems, trifluoroacetic acid (TFA) was normally used as a solvent. Spectra were run on Varian A-60 and/or HA-100 spectrometers.