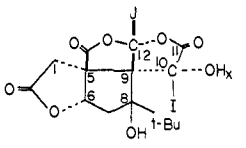

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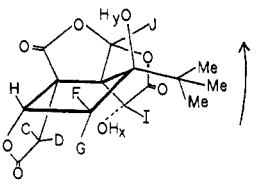

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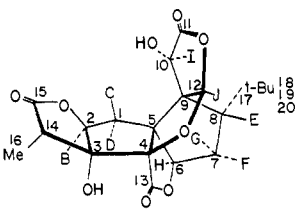

10

In order for the *tert*-Bu group to be sufficiently close to both H_I and H_J in **1** and diacetate **3** (to account for NOE's), the cyclopentane ring must be puckered so that the *tert*-Bu is quasiequatorial,[1f] and of the two possible arrangements, *tert*-Bu cis or trans to H_H, only the trans arrangement can account for the observed J_{HF} and J_{HG} (*ca.* 7 Hz) (see **9**; in the opposite configuration, the cyclopentane would be puckered downward[1f] and adopt a conformation in which these *J* values cannot be both large). The structure thus derived bears a striking resemblance to the diterpenoid ginkgolides, *e.g.*, ginkgolide A, **10**.[1,2] The absolute configuration is based on that of the latter.[1a,e]

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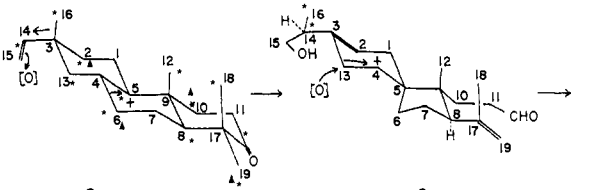
Biosynthesis of Ginkgolide B, Its Diterpenoid Nature, and Origin of the *tert*-Butyl Group

Sir:

Gingko biloba ("icho" in Japanese), an extraordinary plant which has remained unchanged during the past several million years, is the only living species of the order Ginkgoales (ancestry traced back to Jurassic period). The ginkgolides[1] and bilobalide[2] isolated from the root bark and leaves of this tree are not only very unique cage molecules containing three lactone rings, but are still the only natural products having a *tert*-Bu group.[3] Because of their complex framework, it is not immediately obvious to which category of compounds they belong, and ginkgolides have even been classified as being carbohydrates.

In the following we present biosynthetic evidence for their terpenoid origin, the formation of the *tert*-Bu group, and the general biosynthetic route (Scheme I)

Scheme I. Biosynthesis of Ginkgolide B (**1**)[a]



2 **3**

[a] The origins of carbon atoms are denoted as follows: acetate, *; mevalonate, ▲; methionine, ●. The numbering system in **2** corresponds to that in **1**.

which is in accord with the stereochemistry of pertinent chiral centers.

Biosynthetic studies were attempted by tissue culture techniques first developed for *G. biloba* by Tulecke.[4] A total of 11 different media were tested under a variety of growth periods, and although we succeeded in inducing callus growth[5] from both root and shoot tissues, there was no biosynthesis of ginkgolides. On the other hand, usage of embryos[6] was partially successful. Namely, embryos from 5-month old seeds were inoculated in agar medium containing suitable nutrients[5] and [2-^{14}C]mevalonate; several days later the short roots and shoots were separately processed after addition of cold ginkgolide. Although radioactive ginkgolides could indeed be isolated from young roots, the incorporation yield was only 0.01% at the highest (4–10 days after inoculation), and moreover, the method was far too tedious for practical purposes.

Accordingly, the cotton wick method was finally employed (Table I). After suitable incorporation periods, cold ginkgolide B (**1**) (GB, the only detectable ginkgolide under the experimental conditions) was added, and the GB was purified by column chromatography, tlc, and repeated recrystallizations until constant specific activity was attained. In the case of sodium [2-^{14}C]acetate (indicated in Scheme I by *) the final GB obtained had 8.90×10^5 dpm/mmol (40 mg).

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(2) K. Nakanishi, K. Habaguchi, Y. Nakadaira, M. C. Woods, M. Maruyama, R. T. Major, M. Alauddin, A. R. Patel, K. Weinges, and W. Bähr, *J. Amer. Chem. Soc.*, **93**, 3544 (1971).

(3) See the following for a general description of insect repellant properties of *G. biloba:* R. T. Major, *Science*, **157**, 1270 (1967).

(4) W. Tulecke, *Phytomorphology*, 17, 381 (1967), and earlier papers.

(5) K. Habaguchi, to be published elsewhere.

(6) E. Ball, *Amer. J. Bot.*, **46**, 130 (1959).

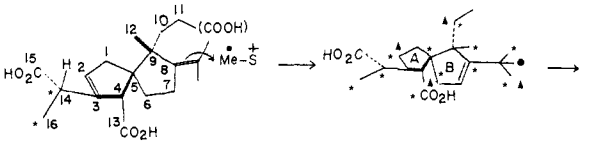
Table I. Incorporation of Labeled Precursors by the Cotton Wick Method [a]

Precursor	No. of plants	Period of administration, days	% incorporation into GB[b]
Sodium [2-^{14}C]acetate (*) 1.0 mCi	120	14	0.0033
[2-^{14}C]MVA (rac) (▲) 0.3 mCi	60	7	0.0048
[Me-^{14}C]Methionine (●) 0.3 mCi	80	5	0.0027

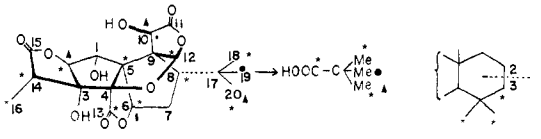
[a] Five-month plants of 20–25 cm height were employed. [b] Based on ginkgolide B (GB) recrystallized to constant specific activity.

Kuhn–Roth oxidation of GB **1**[1b] gave pivalic acid and acetic acid from 16-Me, which were separated by column chromatography and identified by tlc[7] and paper chromatography,[8] coupled with scintillation counting. The radioactive acids were submitted to Schmidt oxidation, the pivalic acid giving *tert*-butylamine (recrystallized to constant specific activity as its 2,4-dinitrophenyl benzoate, mp 182°) and carbon dioxide (trapped as barium carbonate). It was found that the radioactivity was distributed in the ratio of 2.2:1.0 (*tert*-$BuNH_2$ dinitrobenzoate–$BaCO_3$, see **6**). This clearly showed that, assuming a terpenoid origin for the ginkgolides, *the tert-Bu group could not have simply originated by a fission between C-2 and C-3 as in* **7**. The activity in acetic acid was distributed in a ratio of 1.2:1.0 between the methyl and carboxyl groups ($MeNH_2$ dinitrobenzoate–$BaCO_3$) (for discussion, see below.)

Mevalonate (denoted by ▲) was also incorporated (Table I) thus establishing *the terpenoid nature of the ginkgolides*. Acetic acid was not labeled; on the other hand, the pivalic acid was found to contain 95% of the activity expected from one MVA unit, provided the four C_5 units are evenly distributed.[9]



4 **5**



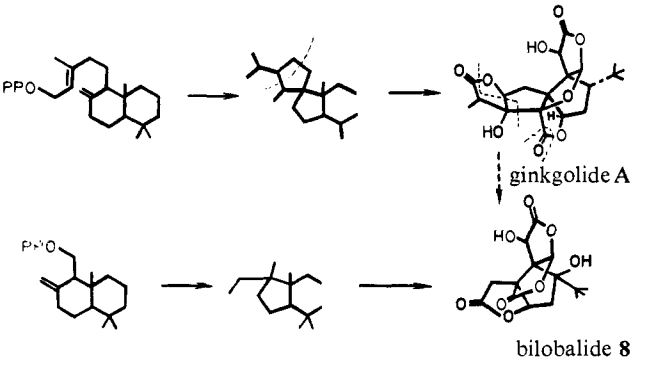
1 **6** **7**

The ginkgolides were next administered with methionine (denoted by ●) in order to clarify the origin of the *tert*-Bu group. As shown in Table I, methionine was incorporated as expected, the radioactivity being exclusively located in the pivalic acid resulting from oxidation of GB.

The evidence mentioned above leads to Scheme I for ginkgolide biosynthesis. The precursor could be an entpimaradienone cation, *e.g.*, **2**, which undergoes three major modifications: (i) migration of the Me group from C-3 to C-14 (ginkgolide numbering is employed in **2**–**5**) which is in agreement with the even distribution of activity in the acetic acid resulting from acetate incorporation; (ii) formation of the characteristic spiro[4.4]nonane moiety with the correct stereochemistry at C-9; and (iii) formation of the *tert*-Bu group initiated by cleavage of the bond adjacent to the *gem*-dimethyl group, a cleavage frequently encountered in terpenoids.[10,11] Intermediacy of a structure such as **4** or **5** is necessary to explain the inversion at C-8 from going to **2** to the ginkgolides **1**.

Clearly bilobalide **8**[2] should be a sesquiterpene. Its biogenesis (Scheme II) can be explained either as

Scheme II. Relation between the Ginkolides (*e.g.*, Ginkolide A) and Bilobalide **8**[a]



[a] The latter can either be derived from GA, by losing the five carbons indicated by dotted lines in the GA structure, or from farnesol.

being a pentanorginkgolide, or a genuine sesquiterpene derivable from farnesyl pyrophosphate in a manner similar to that shown for the ginkgolides.[12]

(7) A. Lynes, *J. Chromatogr.*, **15**, 108 (1964).
(8) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, 23, 1033 (1951).
(9) Although rare, preferential labeling has been observed, *e.g.*, coriamyrtin (sesquiterpene): M. Biollaz and D. Arigoni, *Chem. Commun.*, 633 (1969).
(10) D. Arigoni, D. H. R. Barton, R. Bernasconi, C. Djerassi, J. S. Mills, and R. Wolff, *J. Chem. Soc.*, 1900 (1960); G. H. Whitham, *ibid.*, 2016 (1960).
(11) K. Habaguchi, M. Watanabe, Y. Nakadaira, and K. Nakanishi, *Tetrahedron Lett.*, 3731 (1968).
(12) We acknowledge support by the Ministry of Education (Japan) and USPHS Grant No. CA 11572.

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A Novel Palladium(II)-Catalyzed Cis–Trans Isomerization of Enol Propionates and Vinyl Halides[1]

Sir:

There have been many reports of double bond and cis–trans isomerization catalyzed by metal salts.[2] Mechanisms suggested for these reactions include intermolecular hydride transfer *via* metal hydrides, intramolecular hydride transfer *via* π-allyl hydrides, and reversible π-allyl complex formation.[2c] This communication will describe a Pd(II)-catalyzed cis–trans

(1) Hercules Research Center Contribution No. 1549.
(2) For recent reviews see: (a) N. R. Davies, *Rev. Pure Appl. Chem.*, 17, 83 (1967); (b) E. W. Stern, *Catal. Rev.*, 1, 125 (1967); (c) F. R. Hartley, *Chem. Rev.*, 69, 799 (1969).