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DETECTION OF GINKGOLIDES BY THIN-LAYER CHROMATOGRAPHY[1]

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ABSTRACT.—A method for the analysis of ginkgolides by tlc was developed. It allows for the detection of ginkgolides A, B, and C at the 4-μg level, an 86% increase in sensitivity over previously published methods.

Ginkgo biloba L. is the world's oldest tree species and represents the sole survivor of the family Ginkgoaceae (1). Numerous compounds have been identified from the leaf extracts of *G. biloba*, of which the ginkgolides (diterpenes) appear to possess useful pharmacological properties (2). Standardized extracts of *G. biloba* leaves are marketed by IPSEN Laboratories (France) under the trade name of Tanakan, recommended for the treatment of cerebral vascular disease and senile dementia. Recent studies suggest that ginkgolide B (coded BN 52063) is a potent platelet-activating factor (PAF) antagonist, which may prove useful in the treatment of asthma, endotoxic shock, and graft rejection (3).

Tlc methods for the initial screening of leaf extracts for the presence of ginkgolides remained unsatisfactory due to their lack of sensitivity and specificity (4). For example, the Lobstein-Guth (4) method of detecting ginkgolides by tlc involved the spraying of eluted plates with anhydride reagent followed by heat treatment for 30 min. Under these conditions, ginkgolides emit a faint orange fluorescence under excitation at wavelength of 365 nm. This method suffers from a low detection level (approximately 30 μg for ginkgolide B) and hence is not suitable for detecting ginkgolides in leaf extracts. Repetition of the Lobstein-Guth methodology (4) by our laboratory confirmed these results.

However, replacing the aluminum tlc plates used by Lobstein-Guth with glass tlc plates revealed that the ginkgolides fluoresced much more intensely when viewed through the glass (i.e., back side) of the sprayed tlc plate. The same phenomenon was observed with other spray reagents (e.g., phosphomolybdic acid). This suggests that the spray reagents are masking the fluorescence emitted by the ginkgolides. This observation led to the development of the present method which, instead of treating plates with a spray reagent, simply requires that the developed plate be heated for 35–60 min prior to visualization with uv (365 nm). Using such a method, the limit of detection for ginkgolides A, B, and C was found to be 4 μg. The ginkgolides displayed different R_f values and fluorescence (Table 1), although the fluorescent intensity of ginkgolide C was low compared to that of A and B. Further heating of the plate (1 h) increases the fluorescence of ginkgolide C.

The thin-layer chromatographs of Tanakan and leaf extracts revealed the presence of ginkgolides A and B but not C, which is present in much lower concentrations in ginkgo leaves relative to A and B (5). The solvent system tested allowed for the complete separation of these ginkgolides while maintaining other fluorescent compounds at the origin. This method should prove particularly useful when screening large number of leaf extracts for the presence of the pharmacologically active ginkgolide B.

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TABLE 1. Chromogenic Comparison of Three Ginkgolides.

Ginkgolide	R_f	Color in uv (365 nm)
A	0.52	blue
B	0.47	yellow
C	0.28	pale orange

EXPERIMENTAL

PLANT MATERIAL.—Gingko leaves were obtained in August of 1988 from tree specimens in the Montreal Botanical Garden, Montreal, P.Q., Canada, where a voucher specimen is kept. Leaves were removed from the branches of young trees (15 to 20 years of age) and stored at −80°. Tanakan extract and standards of ginkgolides A, B, and C were obtained from IPSEN Laboratories (France).

EXTRACTION.—Leaf extracts were prepared by boiling 40 g of lyophilized material in H_2O for 20 min, followed by filtration through Whatman No. 1 paper and celite (Hyflo Super-cel). Activated charcoal was added to the filtrate and allowed to stir for 12 h at room temperature. The mixture was centrifuged (1000 g, 15 min), the supernatant discarded, and the charcoal resuspended in 20 ml Me_2CO. After filtration through a glass-fiber filter, the extract was concentrated under reduced pressure to yield an aqueous Me_2CO extract (5).

TLC PROCEDURE.—Stock solutions for each ginkgolide were prepared by dissolving 5 μg of standard per μl of MeOH. Ginkgolide standards (0.8 μl), leaf (8.0 μl) and Tanakan (8.0 μl) extracts were spotted on precoated Si gel K6F 0.25 mm plates (Whatman), which were developed in a presaturated chamber using EtOAc-toluene-Me_2CO-hexane (40:30:20:10) as the solvent. Migration of the solvent front was allowed to proceed to a distance of 10 cm at which point the plates were removed, air-dried, and heated to 150° for 35–60 min prior to visualization under uv light (365 nm).

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