Activity of Bilobalide, a Sesquiterpene from *Ginkgo biloba*, on *Pneumocystis carinii*

CHIARA ATZORI,^{1*} ANTONELLA BRUNO,¹ GUIDO CHICHINO,¹ EZIO BOMBARDELLI,²
MASSIMO SCAGLIA,¹ AND MARIO GHIONE³

Laboratory of Clinical Parasitology, Institute of Infectious Diseases, University of Pavia-IRCCS
San Matteo, 27100 Pavia,¹ Indena, 20141 Milan² and Institute of Medical Microbiology,
Milan University, 20122 Milan,³ Italy

Received 8 December 1992/Accepted 4 May 1993

The sesquiterpene bilobalide, extracted from *Ginkgo biloba* leaves, was tested in vitro and in vivo for the ability to inhibit *Pneumocystis carinii* growth. Bilobalide was inhibitory to trophozoites cultured on human embryonic lung fibroblasts (HEL 299) at approximately the same concentration as trimethoprim plus sulfamethoxazole (lowest effective concentration, 50 µg of bilobalide per ml versus 9/45 µg of trimethoprim-sulfamethoxazole per ml), inducing microscopically detectable morphological changes in the cytoplasm of the parasite. In pharmacologically immunosuppressed Sprague-Dawley rats transtracheally infected with a suspension of about 5×10^6 *P. carinii* trophozoites per ml, the daily intraperitoneal administration of bilobalide (10 mg/kg of body weight for 8 days) lowered the number of organisms by approximately 2 logs (that is, about 99%). There was no apparent toxicity either in uninfected HEL 299 feeder cells or in infected and uninfected animals. These studies suggest that the sesquiterpene bilobalide might be useful for therapy of and prophylaxis against *P. carinii* infections in humans.

Over the past two decades, *Pneumocystis carinii* pneumonia has been recognized as an important opportunistic infection in immunosuppressed patients (7). Among human immunodeficiency virus-infected individuals, *P. carinii* has been the leading cause of disease and death.

Trimethoprim plus sulfamethoxazole (TMP-SMX) and pentamidine have been the most widely used antimicrobial agents for both prophylaxis and therapy, with a high incidence of adverse reactions and toxicity reported. New compounds are needed, and plant products still are sources of potential therapeutic drugs. *Ginkgo biloba*, a member of the traditional Chinese pharmacopeia, was first cited as a medicinal agent some 5,000 years ago and is still in use today. The plant has survived for more than 200 million years, and this extraordinary long-term survival may be due in part to the fact that *G. biloba* synthesizes a number of biologically active compounds, some of which may confer resistance to parasites.

G. biloba contains a particular class of diterpenes and sesquiterpenes, called ginkgolides and bilobalide. Some of the compounds are currently being studied for the treatment of allergic diseases, blood disturbances, and neoplastic and immunological disorders (2, 4, 9, 19, 21, 23, 24, 32, 37, 39, 44, 49). Although recent studies of many plant-derived terpenes have showed that compounds belonging to this class can display antiparasitic (3, 10, 15, 26, 31, 41, 42), antifungal (1, 45), antibacterial (1, 40), antiviral (14), or immunomodulating (8) activities, there has been little interest in bilobalide sesquiterpene. The results of studies of the biologic characteristics of bilobalide in our laboratories indicating activity against *P. carinii* are reported herein.

MATERIALS AND METHODS

TMP-SMX (Bactrim; Roche, Basel, Switzerland) for in vitro tests was added directly to minimal essential medium

(MEM) to a final concentration of 9 µg of TMP per ml and 45 µg of SMX per ml and given after dilution in normal saline to rats at 20 and 100 mg/kg of body weight per day by intraperitoneal injection. A bilobalide (Indena, Milan, Italy)-acetone solution was diluted in distilled water to a final concentration of 6.5 to 500 µg/ml in culture medium and administered intraperitoneally to rats at 1 and 10 mg/kg/day.

Both bilobalide and its hydroxyacetone-water diluent alone (at different concentrations) were tested for toxicity to feeder monolayers and rats.

In vitro studies. Human embryonic lung fibroblasts (HEL 299, ATCC CCL-137) were cultured in 24-well tissue culture plates with MEM (ICN-Flow, Irvin Ayrshire, United Kingdom) containing 10% fetal calf serum and 1% nonessential amino acids. Confluent monolayers were inoculated with lung homogenates obtained from infected rats immunosuppressed with dexamethasone at 0.36 mg/kg/day in drinking water. Infected tissue was ground in MEM, and the inoculum was added to cell cultures in a 1/10 ratio at the same time with diluted drugs to obtain a final concentration of 5×10^5 to 7×10^5 trophozoites per ml. The plates were incubated at 37°C in 5% carbon dioxide. After agitation of the cultures, a 10-µl sample of the culture supernatant from each well was taken for analysis at 1, 5, and 8 days after inoculation, air dried onto a 1-cm² square etched on a glass slide, fixed with methanol, and stained with Giemsa. Each slide was examined microscopically with a 100× objective for quantitation of the organisms. The data are reported as the number of trophozoites per field. The final score for each slide was the mean value of 30 observations; multiplying this number by a factor of 4×10^5 (determined by estimating the number of fields per cm at a magnification of $\times 1,000$) yields the number of organisms per milliliter of culture supernatant. The lowest effective concentration in vitro was determined by adding bilobalide to *P. carinii* in cell cultures at different concentrations (500, 250, 100, 50, 25, 12.5, and 6 µg/ml) and assessing the number of trophozoites at 5 days after infection.

Hydroxyacetone in distilled water at the highest concen-

* Corresponding author.

FIG. 1. Growth curves obtained by plotting the mean number of *P. carinii* trophozoites in Giemsa-stained culture samples of HEL 299 feeder cells taken on days 1, 5, and 8 after infection. ■, bilobalide (50 µg); □, bilobalide (25 µg); ◆, TMP-SMX (54 µg); ◇, untreated *P. carinii* (control); ▲, water solvent.

tration (16%) used was added to the cell cultures to test for the possible activity of residual solvent. Each trial was carried out at least twice.

In vivo studies. Randomly selected groups of eight female Sprague-Dawley *P. carinii*-free rats (Charles River Laboratories, Como, Italy), weighing about 120 g each at the beginning of the trial, were immunosuppressed with dexamethasone (0.36 mg/kg/day) in the drinking water for 6 weeks. Tetracycline (0.5 g/liter) was added to the drinking water to inhibit bacterial infections. After 7 days of immunosuppression, the animals were anesthetized, weighed, and then transtracheally inoculated with a standard suspension of *P. carinii* trophozoites (about $5 \times 10^6/0.2$ ml) in accordance with a previously described protocol (6). Treatment was started the same day that the animals (in groups of eight) were inoculated with the following dosages: bilobalide at 1.0 or 10 mg/kg/day in two doses, TMP-SMX at 20 to 100 mg/kg/day, and the water solvent solution at approximately 16% hydroxyacetone. A group of infected but untreated rats was kept as the control. The drug treatment was carried out for 8 days and then stopped, and the rats were maintained for an additional 5 weeks, at which time the rats were anesthetized with ketamine hydrochloride and sacrificed by cardiac exsanguination, and the lungs were aseptically removed. To evaluate the degrees of infection, lung smears were made and stained with Giemsa to detect trophozoites and active cysts and with toluidine blue to detect active and inactive cysts. The slides were coded and viewed at a magnification of $\times 1,000$ independently by two examiners in a blind format

who used a rating scale with approximately logarithmic intervals. Briefly, the scores were assigned as follows: 5 for >100 microorganisms per field, 4 for 11 to 99 organisms, 3 for 1 to 10 organisms, 2 for 2 to 9 organisms per 10 fields, 1 for 1 organism in 10 or more fields, and 0 for no microorganisms detected in more than 50 fields. The scores from the two observers were averaged to give a single mean value for each group of eight animals.

Comparisons between means were carried out by using the unpaired *t* test. Each experiment was repeated at least three times.

RESULTS

Neither bilobalide nor the hydroxyacetone-water solvent caused damage to HEL 299 feeder cells. No gross toxic effects or signs of cell damage, such as vacuolization, detachment, or inhibition of confluence, could be detected. Bilobalide at 50 µg/ml was as inhibitory to *P. carinii* growth as TMP-SMX (Fig. 1).

The effects of bilobalide were evident also by the morphologic alterations in the cytoplasm of *P. carinii* organisms, which appeared enlarged, dull, and hazy on the eighth day of incubation with a drug (Fig. 2A), while untreated organisms maintained a normal appearance (Fig. 2B).

In vivo, treatment with bilobalide at 10 mg/kg induced a statistically significant decrease in the parasite burden compared with that in untreated rats, and the effect was confirmed by comparison of bilobalide- and TMP-SMX-treated

FIG. 2. Microscopic features of cultured *P. carinii* trophozoites 8 days after inoculation. (A) *P. carinii* incubated with bilobalide (50 $\mu\text{g/ml}$); (B) untreated controls.

TABLE 1. Infectivity scores for rats transtracheally infected with *P. carinii* and treated with bilobalide, water solvent, or TMP-SMX and untreated controls^a

Treatment (drug amt [mg/kg])	Mean weight (g) of rat at:			Infectivity score with:	
	Infection	Week 4	Week 6	Giemsa stain	Toluidine blue
TMP-SMX (20 to 100)	102	94	96	1.8 ± 0.3	1.6 ± 0.1
Bilobalide (1)	109	67	63	4.7 ± 0.2	4.2 ± 0.3
Bilobalide (10)	106	87	73	2.8 ± 0.4	2.6 ± 0.3
Water solvent	103	68	65	4.7 ± 0.2	4.3 ± 0.2
None (untreated control)	107	72	60	4.8 ± 0.1	4.3 ± 0.4

^a Infectivity score data are expressed as the mean (± standard deviation) of 10 observations for each smear of rat lung (see the text). The initial rat weight at the beginning of immunosuppression was 120 g; mean weights were scored at the moment of infection, four weeks after infection, and at the moment of sacrifice (week 6). The scores for the bilobalide (10 mg/kg) and TMP-SMX (20 to 100 mg/kg) treatments are statistically significantly different from those for the untreated control ($P < 0.001$).

groups (Table 1). The decrease in the score with Giemsa stain from 4.8 ± 0.1 to 2.8 ± 0.4 represents a 2-log (that is, about 99%) reduction in organisms. At the end of the sixth week of the observation period, the animals treated with TMP-SMX (20 to 100 mg/kg) or bilobalide (10 mg/kg) retained the glossy fur indicative of normal rats even though they had lost weight, while, in contrast, untreated animals and those treated with 1 mg of bilobalide per ml or the solvent-water mixture had the lowest weights, ruffled fur, hunched backs, often cyanosis of the tail, and a general appearance of disease.

DISCUSSION

The data show that bilobalide, a sesquiterpene extracted from *G. biloba* leaves, inhibits *P. carinii* growth at 50 µg/ml in vitro or at 10 mg/kg in vivo and is therefore a potentially useful new drug for the treatment of and prophylaxis against *P. carinii* pneumonia.

In ancient times, many properties were attributed to the extracts of *G. biloba* leaves. Pharmacological investigations have recently defined some of their characteristics by showing that they possess potent antagonistic properties against platelet-activating factor, an inflammatory mediator structurally characterized as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (24). Furthermore, the screening of polar and lipophilic fractions of the extracts commonly used in therapy recently confirmed that the effects were largely due to the lipophilic class of these rare terpenes (22, 23, 33, 46, 48). Bilobalide, as well as other sesquiterpenes, represents a challenge for the organic chemist and biologists as well, owing to a peculiar cage structure and its potential activity. Because these products are present in *G. biloba* leaves in light-sensitive (16), small amounts (<0.01%), many attempts at synthesis have been made in the last 15 years to obtain the compounds by alternative methods. Recently, the synthesis of bilobalide and other sesquiterpenes was successfully performed by means of an elegant and sophisticated pathway (11, 12); however, synthesis is not yet practical on an industrial scale (13), and at present, the natural source remains the sole one for preliminary experiments.

The chemical structure of bilobalide, a sesquiterpene extracted from *G. biloba*, incorporates a tert-butyl group and five fused cyclopentanoid rings, among which are three gamma-lactone units and one cyclopentane unit; the *cis*-

fused rings F, A, D, and C are folded in such a way that a semispherical cavity is formed. In relative molecular terms, this cavity is sufficiently large to host other inorganic or organic groups; this can explain the chemical behavior of this amphiphilic molecule and its biological activity.

Besides bilobalide, another sesquiterpene, the antimalarial agent artemisinin (Qinghaosu), has been shown to possess, at least in vitro, inhibitory activity against *P. carinii*; no data on its action in vivo are available (30). It is possible that the effect of bilobalide on *P. carinii* may be the prevention of the reported interaction of *P. carinii* with the membranes of alveolar epithelium cells in vivo and with the surfaces of cultured lung cells (28, 29, 34, 35, 43). The presence of the lipotropic sesquiterpene molecules may be inhibitory to normal attachment of trophozoites. Another hypothesis involves the known effect of *G. biloba* extracts on the cytoskeleton function. Agents known to impair cytoskeleton function, such as cytochalasin, colchicine, and some benzimidazole derivatives, depress the adherence of *P. carinii* to 549 and HEL 299 cultured cells (5, 27). *G. biloba* extracts have been reported to interfere with phenomena involved in cytoskeleton function such as mast cell degranulation (49), in stabilization of lysosomes (47) or collagen fibrils (18), and in cell damage triggered by free radicals (4, 33, 39). Whatever the mechanism(s) of action, bilobalide affects *P. carinii* growth without signs of cytotoxicity to feeder layers even after 8 days of culture in the presence of the sesquiterpene up to a concentration of 500 µg/ml, i.e., 10-fold higher than the minimum effective concentration in vitro.

In animals, bilobalide caused an infectivity score reduction of approximately 2 logs (that is, about 99%) and was comparable to the most effective anti-*P. carinii* drug regimen known. The effectiveness is very well demonstrated in this model, as bilobalide was given for only 8 days rather than the usually reported 14 or 21 days in therapy protocols or 5 to 6 weeks in prophylaxis protocols.

In conclusion, the data reported here show that bilobalide inhibits the growth of *P. carinii* in vitro and in vivo so that bilobalide and possibly other sesquiterpene compounds deserve further investigation as potential new drugs for the treatment of *P. carinii* pneumonia. The potential is great for further study since *G. biloba* extracts containing bilobalide have already been used in medicines for humans (17, 20, 21, 23, 25, 36, 38) without evidence of side effects.

ACKNOWLEDGMENTS

This work was partially supported by the Italian Ministry of Health, AIDS Project, 1993.

REFERENCES

1. Anke, H., and O. Sterner. 1991. Comparison of the antimicrobial and cytotoxic activities of twenty unsaturated sesquiterpene dialdehydes from plants and mushrooms. *Planta Med.* 57:344-346.
2. Attella, M. J., S. W. Hoffman, M. J. Stasio, and D. G. Stein. 1989. *Ginkgo biloba* extract facilitates recovery from penetrating brain injury in adult male rats. *Exp. Neurol.* 105:62-71.
3. Balu, N., J. V. Thomas, and S. V. Bath. 1991. Monoterpenic fragment analogues of aplasmomycin as potential antimalarial. *J. Med. Chem.* 34:2821-2823.
4. Barth, S. A., G. Inselmann, R. Engemann, and H. T. Heide-mann. 1991. Influence of *Ginkgo biloba* on cyclosporin A induced lipid peroxidation in human liver microsomes in comparison to vitamin E, glutathione and *N*-acetylcysteine. *Biochem. Pharmacol.* 41:1521-1526.
5. Bartlett, M. S., T. D. Edlind, M. M. Durkin, M. M. Shaw, S. F. Queneer, and J. W. Smith. 1992. Antimicrotubule benzimidazoles inhibit in vitro growth of *Pneumocystis carinii*. *Antimi-*

- cro. Agents Chemother. 36:779-782.
6. Bartlett, M. S., J. A. Fishman, S. F. Queener, M. M. Durkin, M. A. Jay, and J. W. Smith. 1988. New rat model of *Pneumocystis carinii* infection. J. Clin. Microbiol. 26:1100-1102.
7. Bartlett, M. S., and J. W. Smith. 1991. *Pneumocystis carinii*, an opportunist in immunocompromised patients. Clin. Microbiol. Rev. 4:137-149.
8. Bourguet-Kondracki, M. L., A. Longeon, E. Morel, and M. Guyot. 1991. Sesquiterpene quinones as immunomodulating agents. Int. J. Immunopharmacol. 13:393-399.
9. Braquet, P., and D. Hosford. 1991. Ethnopharmacology and the development of natural PAF antagonists as therapeutic agents. J. Ethnopharmacol. 32:135-139.
10. Chiari, E., A. B. De Oliveira, D. S. Raslan, A. A. Mesquita, and K. G. Tavares. 1991. Screening in vitro of products against blood forms of *Trypanosoma cruzi*. Trans. R. Soc. Trop. Med. Hyg. 85:372-374.
11. Corey, E. J. 1988. Enantioselective total synthesis of bilobalide and C15 ginkgolide. Tetrahedron Lett. 29:3423-3426.
12. Corey, E. J., M. C. Kang, M. C. Desai, A. K. Ghosh, and I. N. Houpi. 1988. Total synthesis of racemic ginkgolide B. J. Am. Chem. Soc. 110:649-651.
13. Crimmins, M. T., D. K. Jung, and J. L. Gray. 1992. A total synthesis of ($\pm$) bilobalide. J. Am. Chem. Soc. 114:5445-5447.
14. De Tommasi, N., C. Pizzi, C. Conti, N. Orsi, and M. L. Stein. 1990. Structure and in vitro antiviral activity of sesquiterpene glycoside from *Calendula arvensis*. J. Nat. Prod. 53:830-835.
15. Fernex, M., C. Jequet, M. L. Miktelholzer, R. Reber, and D. Sturchler. 1991. Neue Medikamente für die Behandlung der Malaria tropica. Schweiz. Rundsch. Med. Prax. 80:67-71.
16. Flesch, V., M. Jaques, L. Cosson, B. P. Teng, V. Petiard, and J. P. Balz. 1992. Relative importance of growth and light level on terpene content of *Ginkgo biloba*. Phytochemistry 31:1941-1945.
17. Grassel, E. 1992. Effect of *Ginkgo biloba* extract on mental performance. Double-blind study using computerized measurement conditions in patient with cerebral insufficiency. Fortschr. Med. 110:73-76.
18. Herbage, D., R. Willems, and F. Flandin. 1991. Evaluation of extracts of *Ginkgo biloba* and its constituents on the fibrillogenesis and on stability of collagen fibrils in vitro. Phlebologie 44:873-880.
19. Itokawa, H., N. Totsuka, K. Nakahara, M. Maezuru, K. Takeya, M. Kondo, M. Inamatsu, and H. Morita. 1989. A quantitative structure activity relationship for antitumor activity of long chain phenols from *Ginkgo biloba*. Chem. Pharm. Bull. 37: 1619-1621.
20. Jung, F., C. Mrowietz, H. Kiesewetter, and E. Wenzel. 1990. Effect of *Ginkgo biloba* on fluidity of blood and peripheral microcirculation in volunteers. Arzneim.-Forsch. 40:589-593.
21. Kleijnen, J., and P. Knipschild. 1992. *Ginkgo biloba*. Lancet 340:1136-1139.
22. Koltringer, P., and O. Eber. 1989. Die kollageninduzierte Thrombozytenaggregation unter parenteraler *Ginkgo biloba* Therapie. Wien Med. Wochenschr. 139:92-94.
23. Koltringer, P., O. Eber, G. Klima, W. Rothlauer, P. Wakoning, W. Langsteger, and P. Lind. 1989. Die Mikrocirkulation unter parenteraler *Ginkgo biloba* Extrakt Therapie. Wien Klin. Wochenschr. 101:198-200.
24. Kurihara, K., A. J. Wardlaw, R. Moqbel, and A. R. Kay. 1989. Inhibition of platelet-activating factor-induced chemotaxis and PAF binding to human eosinophils and neutrophils by the specific ginkgolide-derived PAF antagonist BN 52021. J. Allergy Clin. Immunol. 83:83-90.
25. Lacomblez, L., D. Warot, T. Tarrade, and A. J. Puech. 1990. Psychopharmacological effects of *Ginkgo biloba* extract EGB-761 in healthy volunteers. Eur. J. Pharm. 183:1460.
26. Lee, I. S., and C. D. Hufford. 1990. Metabolism of antimalarial sesquiterpene lactones. Pharmacol. Ther. 48:345-355.
27. Limper, A. H., and J. W. Martin II. 1989. *Pneumocystis carinii*: concanavalin A alters parasite adherence to cultured lung cells. Am. Rev. Respir. Dis. 139:A127.
28. Limper, A. H., and J. W. Martin II. 1990. *P. carinii*: inhibition of lung cell growth mediate by parasite attachment. J. Clin. Invest. 85:391-397.
29. Long, E. G., J. S. Smith, and J. L. Meier. 1986. Attachment of *Pneumocystis carinii* to rat pneumocytes. Lab. Invest. 54:609-615.
30. Merali, S., and S. R. Meshnick. 1991. Susceptibility of *Pneumocystis carinii* to artemisinin in vitro. Antimicrob. Agents Chemother. 35:1225-1227.
31. Ou-Yang, K., E. C. Kug, J. J. Marr, and R. L. Berens. 1990. Inhibition of growth of *Toxoplasma gondii* by Qinghaosu and derivatives. Antimicrob. Agents Chemother. 34:1961-1965.
32. Pincemail, J., M. Dupuis, C. Nasr, P. Hans, M. Haag-Berrurier, R. Anton, and C. Deby. 1989. Superoxide anion scavenging effect and superoxide dismutase activity of *Ginkgo biloba* extract. Experientia 45:708-712.
33. Porsolt, R. D., P. Martin, A. Lenegre, S. Fromage, and K. Drieu. 1990. Effects of an extract of *Ginkgo biloba* EGB-761 on learned helplessness and other models of stress in rodents. Pharmacol. Biochem. Behav. 36:963-972.
34. Pottraz, S. P., and J. W. Martin II. 1990. Role of fibronectin in *Pneumocystis carinii* attachment to cultured lung cells. J. Clin. Invest. 85:351-356.
35. Pottraz, S. P., and W. J. Martin II. 1990. Mechanism of *Pneumocystis carinii* attachment to cultured rat alveolar macrophages. J. Clin. Invest. 86:1678-1683.
36. Rai, G. S., C. Shovlin, and K. A. Wesnes. 1991. A double blind, placebo controlled study of *Ginkgo biloba* extract ("takanan") in elderly outpatients with mild to moderate memory impairment. Curr. Med. Res. Opin. 12:350-355.
37. Remassamy, C., F. Clostre, Y. Christen, and J. Costentin. 1990. Prevention by a *Ginkgo biloba* extract (GBE 761) of the dopaminergic neurotoxicity of MPTP. J. Pharm. 42:785-789.
38. Schneider, B. 1992. *Ginkgo biloba* extract in peripheral arterial disease. Meta-analysis of controlled clinical trials. Arzneim.-Forsch. 42:428-436.
39. Szabo, M. E., M. T. Droy-Lefaux, M. Doly, and P. Braquet. 1991. Free radical mediated effects in reperfusion injury with superoxide dismutase and EGB-761 in rat retina. Ophthalmic Res. 23:225-234.
40. Takazawa, H., and S. Kashino. 1991. Incarnal. A new antibacterial sesquiterpene from basidiomycetes. Chem. Pharm. Bull. 39:555-557.
41. Tan, R. X., Z. J. Jia, J. Jakupovic, F. Bohlmann, and S. Huneck. 1991. Sesquiterpene lactones from *Artemisia rutifolia*. Phytochemistry 30:3033-3035.
42. Tripathi, R., G. P. Dutta, and R. A. Vishwakarma. 1990. Gametocidal activity of the antimalarial alpha-beta arteether against *Plasmodium cynomolgi* B. Am. J. Trop. Med. Hyg. 43:571-575.
43. Walzer, P. D. 1986. Attachment of microbes to host cells: relevance of *Pneumocystis carinii*. Lab. Invest. 54:589-590.
44. Warot, D., L. Lacomblez, P. Danjou, E. Weiller, C. Payan, and A. J. Puech. 1991. Comparison des effets d'extraits de *Ginkgo biloba* sur les performances and memoire chez le sujet sain. Therapie 46:33-36.
45. Watanabe, N., M. Yamagishi, T. Mizutani, H. Kondon, J. Omura, K. Hanada, and K. Kushida. 1990. CAF 603: a new antifungal carotene sesquiterpene. Isolation and structure elucidation. J. Nat. Prod. 53:1176-1181.
46. Wattiaux, R., F. Dubois, and S. Wattiaux-de Coninck. 1991. Effect de differents extraits de *Ginkgo biloba* sur la reponse des lysosomes aux radicaux libres et au choc osmotique. Phlebologie 43:753-761.
47. Wattiaux-de Coninck, S., C. Thibout, M. M. Gouze, and R. Wattiaux. 1989. Effect de l'extrait de *Ginkgo biloba* sur la stabilisation des lysosomes. Phlebologie 42:667-672.
48. Winter, E. 1991. Effects of an extracts of *Ginkgo biloba* on learning and memory on mice. Pharmacol. Biochem. Behav. 38:109-114.
49. Zhang, H., L. Xu, Q. Jin, and P. Cheng. 1990. Anti-anaphylactic pharmacological action of water soluble constituents of *Ginkgo biloba* emisperm. Chung. Kuo. Chung. Yao. Tsa. Chih. 15:496-497, 513.