

COMPONENTS OF *GINKGO BILOBA* LEAF WAX

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## INTRODUCTION

The only surviving species of the Ginkgoales is the tree *Ginkgo biloba* L., which retains many primitive characteristics and can be described as a 'living fossil' [1]. The remarkable ability to survive in changing environments, together with the species' obvious resistance to external aggression, has been of interest to biologists and chemists for many years. Different organs have been chemically analysed, such as seeds [2–5], leaves [1, 4–9] and roots [4], with the idea of establishing a correlation of resistance with chemical composition. Together with this is the possibility of using chemical composition as a measure of primitive characters.

In a plant, the epicuticular waxes represent the first line of defense against external attack [10] or environmental aggression [11]. In this study we report the main components of the leaf wax of this species.

## RESULTS AND DISCUSSION

The first analysis of *G. biloba* leaf wax was carried out by Ageta [6], without the benefit of chromatographic techniques. Ageta reported wax yields of 0.7–1.0%, of which 15% was considered to be esters. Nonacosane, 10-nonacosanone, 10-nonacosanol and 1-octacosanol were reported present. The secondary alcohol fraction was analysed more recently by Holloway *et al.* [9], who reported the presence of other secondary alcohols.

As could be expected, using GLC techniques all fractions were found to be more complex. Hydrocarbons (19% of the total wax) were mainly normal odd-carbon numbered homologues, with C<sub>27</sub>, C<sub>25</sub> and C<sub>29</sub> predominating. Esters (18%) were mainly C<sub>38</sub>, C<sub>40</sub> and C<sub>42</sub> components. Their principal acids were C<sub>22</sub> and C<sub>20</sub>, and their alcohols mainly 1-octacosanol (Table 1). The free alcohol fraction (27%) was almost pure 10-nonacosanol, in general accordance with Holloway's results.

The composition of the free secondary alcohol fraction was established by reduction to the *n*-alkane, and by GC-MS analysis of the corresponding ketones. The MS results showed that the main ketone obtained was 10-nonacosanone (M<sup>+</sup> 422; *m/e* 421, 311, 310, 296, 295, 250, 239, 197, 183, 171, 170, 156, 155) [12], together with minor amounts of 10-heptacosanone (M<sup>+</sup> 394; *m/e* 393, 295, 283, 282, 171, 170, 155) and 13-nonacosanone (M<sup>+</sup> 422; *m/e* 269, 268, 256, 213, 212, 197). The <sup>13</sup>C NMR spectrum of the purified main component was consistent with that expected for 10-nonacosanol:  $\delta$  (in ppm from TMS): 72.1 (C<sub>10</sub>); 37.6 (C<sub>9,11</sub>); 31.9 (C<sub>3,27</sub>); 29.7 (C<sub>5,6,7,13–25</sub>); 29.4 (C<sub>4,26</sub>); 25.7 (C<sub>8,12</sub>); 22.7 (C<sub>2,28</sub>); 14.1 (C<sub>1,29</sub>).

Many primitive plants (*Cycas revoluta* [13], *Ephedra gerardiana* [7], *Chamaecyparis obtusa* [14], *Ch. lawsoniana* [9], *Diplopterium glaucum* [15], *Picea sitchensis*, *P. pungens* and *Agathis australis* [9]) have 10-nonacosanol as the main component of the free alcohol fraction.

Table 1. Composition % of hydrocarbon, ester and free alcohol fractions of *G. biloba* leaf wax

Homologues	Hydrocarbons	Hydrolysis products of esters		Secondary alcohols
		Acids	Alcohols	
16	—	4	—	—
18	—	5	—	—
20	—	30	—	—
22	3	42	1	—
23	6	1	11	—
24	8	10	3	—
25	15	1	7	—
26	12	2	12	—
27	25	1	3	7*
28	9	—	42	3
29	10	—	2	90†
30	7	—	14	—
31	5	—	—	—
Unidentified	0	4	5	0

\* Main component is 10-heptacosanol.

† Main component is 10-nonacosanol (91%), minor component 13-nonacosanol (9%).

In some of these reports, the presence of other minor components has not been established [7, 13–15]. The epicuticular waxes of some of the earlier [16] flowering plants also show the presence of 10-nonacosanol. The Rosaceae [9, 12, 17], Ranunculaceae [9] and Papaveraceae [9, 11, 18, 19] have this compound as the main component of the free alcohols (73–99% of the fraction), accompanied by much smaller amounts of other nonacosanols and other members of the homologue series [9, 12, 17]. 10-Nonacosanol also is the main component of the free alcohols in one species of Tropaeolaceae [9], one Anacardiaceae [9] and one Liliaceae [9]. In rose petals, 9-hentriacontanol dominates [12].

In the Cruciferae either 15-nonacosanol [20, 31] or 14-nonacosanol [9, 21] predominate. The presence of 10-nonacosanol in this group [17] has not been confirmed. 16-Hentriacontanol is the main free alcohol in *Pisum sativum* leaves [9, 22, 23]. The Cruciferae are supposed to be a more evolved group [16], derived from the Ranunculaceae, and the Fabaceae from the Rosales [16]. In the Monocots, *Tulipa* has been reported to have free secondary alcohols [9]. The esterified 2-alkanols reported for barley waxes [24] cannot be classified together with the other secondary alcohols.

## EXPERIMENTAL

Column chromatography was carried out as described previously [25, 26] (Kieselgel Woelm: 0.063–0.200 mm), using

hexane and hexane- $\text{CHCl}_3$  mixtures of increasing polarity. TLCs were run on Si Gel G, using petrol- $\text{Et}_2\text{O}$ -HOAc (45:5:1) as solvent. GLC was carried out using the usual procedures [26, 27] on a FID instrument. Parafilm was used as standard for the hydrocarbon identifications [28]. The fatty acid Me esters and 1-alkanols present in the ester fraction were obtained by methanolysis with  $\text{BF}_3$ -MeOH [29]. The Me esters and 1-alkanol acetates were identified by comparison with authentic samples. GC-MS were run on a quadropole instrument.

*Preparation of wax.* *G. biloba* leaves (1 kg) were collected in Montevideo, Uruguay (March, 1977) [30]. The leaves were extracted by immersion in petrol (ANCAP 60-80°, redistilled) for 15 sec to avoid simultaneous extraction on non-superficial lipids. The wax (0.93 g; 0.9 %) was recovered by evapn of solvent.

*Analysis of the secondary alcohol fraction.* The eluate obtained using hexane- $\text{CHCl}_3$  (4:1) mixture was evapd. The residue could be crystallized from hexane-MeOH. The purified product was acetylated to give a single product (GLC). The original residue was converted into the corresponding hydrocarbons by treatment with  $\text{I}_2$ -red P in a sealed tube, followed by reduction with Zn/HCl and extraction [12]. The resulting hydrocarbons were analysed by GLC. The original residue was converted to the corresponding ketones by treatment with  $\text{CrO}_3$  in HOAc [12].

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