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FLAVONOID GLYCOSIDES AND THE CHEMOSYSTEMATICS OF *EUCALYPTUS CAMALDULENSIS*

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Key Word Index *Eucalyptus camaldulensis*; *E. occidentalis*; Myrtaceae: flavone and flavonol glycosides; chemosystematics.

Abstract–Leaf flavonoid glycosides of *Eucalyptus camaldulensis* were identified as kaempferol 3-glucoside and 3-glucuronide; quercetin 3-glucoside, 3-glucuronide, 3-rhamnoside, 3-rutinoside and 7-glucoside; apigenin 7-glucuronide and luteolin 7-glucoside and 7-glucuronide. Two chemical races were observed based on the flavonoid glycosides. These races correspond to the northern and southern populations of species growing in Australia. The Middle Eastern species examined were found to belong to the southern Australian chemical race. The major glycosides of *E. occidentalis* proved to be quercetin and myricetin 3-glucuronide.

INTRODUCTION

In Egypt, *Eucalyptus camaldulensis* Dehnh. and *Casuarina* species are the most widely planted trees. *E. camaldulensis* was introduced into Egypt nearly a century ago from Australia, and has since shown great adaptability to climatic and edaphic conditions. A tree improvement program is presently underway at Alexandria University, which involves the introduction of new *E. camaldulensis* provenances. A preliminary survey indicates that some of the old plantations are superior to their descendents. Therefore it is essential to know the origin of the initial material, in order that seed of the same provenance(s) may be imported.

Eucalyptus camaldulensis is known to be a botanically variable species [1] and this is reflected in the variety of the polyphenol aglycones which have been detected [2]. Hillis [2] also noted differences in flavanol glycosides amongst different samples of *E. camaldulensis* and suggested that it may be possible to ascertain from an examination of the aglycones and glycosides the original provenances of those plants of *E. camaldulensis* which are growing vigorously in countries outside Australia.

The present work was undertaken to examine the chemical variation in *E. camaldulensis* and to determine the probable origin of some local plantations.

RESULTS AND DISCUSSION

A wide range of flavonoids have been reported in *Eucalyptus* species, besides stilbenes and other phenolics. The flavonoids found include flavonol, flavanones and 3-hydroxyflavanones as well as proanthocyanidins. Proanthocyanidins were mainly detected in the wood [3, 15]; however, they are also reported in the leaves [2]. Flavanones are also present in both leaf and wood tissue. Thus aromadendrin (dihydrokaempferol) was identified in the kino of *E. calophylla* [4], *E. corymbosa* [5], *E. hemiphloia* [6] and *E. lanceolata* [7] and the leaves of *E. flocktoniae* [10], *E. salubris* [10] and *E. sideroxylon* [11]. Aromadendrin 7-methyl ether was characterized in the kino of *E. citriodora* [8] and *E. maculata* [9]. The flavanone sakuranetin was detected in the kino of *E. calophylla* [4], naringenin in the kino of *E. maculata* [9] and pinocembrin, alpinetin and dimethoxypinocembrin in the leaves of *E. sieberi* [12]. The flavonol glycosides identified in *Eucalyptus* species are the 3-glucosides, 3-rhamnosides and 3-rutinosides of kaempferol [11], quercetin [10, 11, 13, 14] and myricetin [10].

In the present study, the leaves of a number of different plants of *E. camaldulensis* were investigated: five samples from Egypt, one from Israel and twenty-nine from Australia, all from different locations (see Table 1 and Fig. 1). The major flavonoid was quercetin 3-glucuronide in all the samples examined. This is in agreement with the results of Hillis [2, 16], who found quercetin as the major aglycone in all the samples he surveyed. On the basis of the present survey of flavonoid glycosides the plants may be divided into two chemical races a and b. The flavonoid glycosides present in both races are: quercetin 3-glucuronide, 3-rutinoside and 7-glucoside and kaempferol 3-glucuronide. In addition, race a also contains small amounts of apigenin 7-glucuronide, luteolin 7-glucuronide and 7-glucoside. Race b on the other hand, contains quercetin 3-glucoside and kaempferol 3-glucoside (mainly traces), instead of the flavone glycosides. In addition, four samples of race b also contain small amounts of quercetin 3-rhamnoside. No aromadendrin or taxifolin could be detected in any samples before or after acid hydrolysis. Simple phenolics were consistently present in all samples especially gallic and ellagic acids, but in varying concentrations. Traces of proanthocyanidins were present in some samples; however, their distribution did not seem to fit in with any other phenolic patterns.

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Table 1. Flavonoid glycosides of *E. camaldulensis* and *E. occidentalis*

	Provenance number*	Origin of provenance†	Chemical race	Flavonoid glycosides‡										
				Apigenin 7-glucuronide	Luteolin 7-glucoside	Luteolin 7-glucuronide	Kaempferol 3-glucoside	Kaempferol 3-glucuronide	Quercetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-rhamnoside	Quercetin 3-rutinoside	Quercetin 7-glucoside	Myricetin 3-glucuronide
E. camaldulensis	6869	A	a	t	t	t	– –	+ +	– –	+ + +	– –	+	+ +	– –
	8031	A	a	t	t	t	– –	+ + +	– –	+ + +	– –	+	+ +	– –
	10574	A	a	t	t	t	– –	+ + +	– –	+ + +	– –	+	+ +	– –
	7116	A	a	t	t	t	– –	+ +	– –	+ + +	– –	+	+ +	– –
	10576	A	a	t	t	t	– –	+	– –	+ + +	– –	+	+ +	– –
	10531	A	a	t	t	t	– –	+ + +	– –	+ + +	– –	+	t	– –
	6788	A	a	+	t	t	– –	+ +	– –	+ + +	– –	+	+	– –
	7037	A	a	+	t	+	– –	+	– –	+ + +	– –	+	+ +	– –
	7046	A	a	+	t	t	– –	+ + +	– –	+ + +	– –	+	+ +	– –
	10913	A	a	+	t	t	– –	+ + +	– –	+ + +	– –	+	+ +	– –
	6953	A	a	t	t	t	– –	+ + +	– –	+ + +	– –	+	+ +	– –
	6948	A	a	t	t	t	– –	+ + +	– –	+ + +	– –	+	+	– –
	10494	A	b	–	–	–	t	+ +	+	+ + +	– –	+	+	– –
	6870	A	b	–	–	–	+	+ +	+ +	+ + +	– –	+	+ +	– –
	7466	A	b	–	–	–	+	+ +	+	+ + +	– –	+	+	– –
	6958	A	b	–	–	–	+	+ +	+ +	+ + +	– –	+	+	– –
	6990	A	b	–	–	–	+	+	+	+ + +	– –	+	t	– –
	6955	A	b	–	–	–	+	+ +	+	+ + +	– –	t	t	– –
	6843	A	b	–	–	–	+	+ +	+	+ + +	+	+	+	– –
	6844	A	b	–	–	–	t	+	+	+ + +	– –	+	+	– –
	6966	A	b	–	–	–	+	+ +	+ +	+ + +	– –	+	+	– –
	10666	A	b	–	–	–	+	+ +	+ +	+ + +	– –	+	+	– –
	10659	A	b	–	–	–	+	+ +	+	+ + +	– –	+	+	– –
	6980	A	b	–	–	–	t	+	+	+ + +	– –	+	t	– –
	6991	A	b	–	–	–	+	+ +	+	+ + +	– –	+	+	– –
	8960	A	b	–	–	–	?	+	t	+ + +	– –	+	+	– –
	10886	A	b	–	–	–	+	+ +	+ +	+ + +	+	+	+ +	– –
	11340	A	b	–	–	–	t	+ +	+	+ + +	– –	+	+	– –
	10885	A	b	–	–	–	+	+ + +	+	+ + +	– –	+	+	– –
	1	E§	b	–	–	–	+	+ +	+	+ + +	– –	+	+ +	– –
	2	E§	b	–	–	–	t	+	+	+ + +	+	+	+	– –
	3	E§	b	–	–	–	t	+ +	+	+ + +	– –	+	t	– –
	4	E§	b	–	–	–	+	+	+	+ + +	+	+	?	– –
	5	E§	b	–	–	–	+	+ +	+	+ + +	– –	+	t	– –
	6	I§	b	–	–	–	+	+ +	+	+ + +	– –	+	+	– –
E. occidentalis	9902	A	–	–	–	–	–	t	– –	+ + +	– –	+	+	+ +
	9903	A	–	–	–	–	– –	t	– –	+ + +	– –	+	+	+ +

* See Fig. 1 for exact location.
† A = Australia, E = Egypt, I = Israel.
‡ + + + = major, t = traces, – = absent.
§ See Experimental for exact location.

The two chemical races a and b correspond very clearly to the "Northern" and "Southern" populations (Fig. 1). The existence of two chemical races in *E. camaldulensis* is not surprising since Banks and Hillis [16] indicated the existence of six groups based on aglycone patterns, which fell more or less into northern and southern divisions. Several authors recognised a northern and southern form of *E. camaldulensis* based on morphological and physiological features [17–19] but the boundaries between the northern and southern forms have not been clearly defined. Approximate dividing lines have been suggested which vary from 20°S to 32°S [19]. From the present study

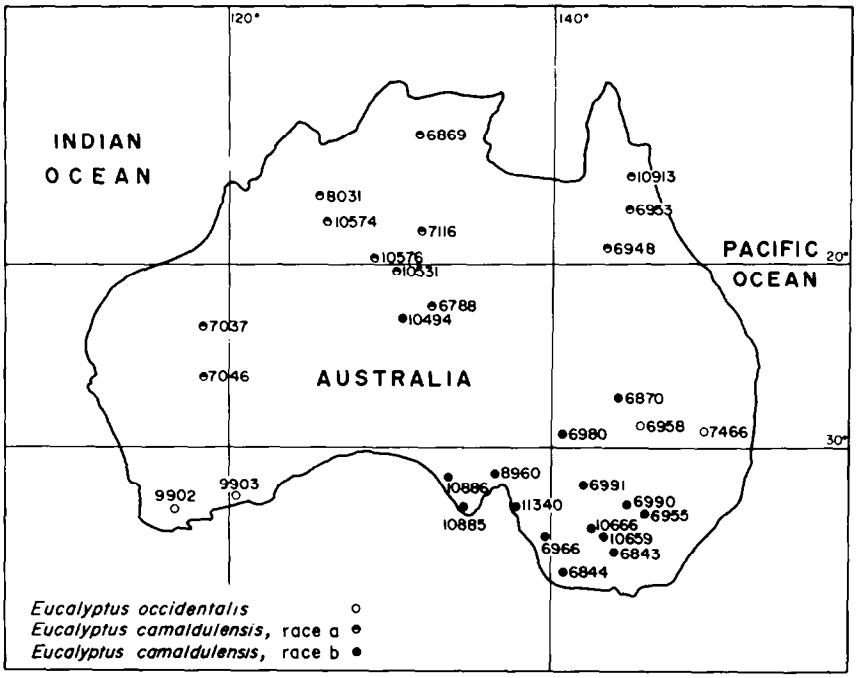

Fig. 1. Location of Australian provenances of *E. camaldulensis* and *E. occidentalis* examined.

it would be difficult to define the exact dividing boundaries thus supporting Pryor's and Byrne's [17] conclusion that *E. camaldulensis* is one distinct species with northern and southern populations which were clearly distinguishable only at their extremes.

The six local samples of *E. camaldulensis* (five from Egypt, one from Israel) (see Experimental for exact locations) were found to belong to race b, which is characteristic of S. Australian provenances (Table 1). This is in agreement with the results of Karschon [18, 20] who concluded that eucalyptus growing in Israel was introduced from S. Australia, a conclusion partially confirmed by the phytochemical study of Banks and Hillis [16]. Furthermore, Turnbull [1] pointed out that practically all the seed for plantations throughout the world originated from the forests along the Murray River or from inland areas in New South Wales.

The flavonoids of two samples of *E. occidentalis* Endl. were also examined (see Table 1 and Fig. 1). The major compounds identified are quercetin 3-glucuronide and myricetin 3-glucuronide. Lesser amounts of quercetin 3-rutinoside and quercetin 7-glucoside were also detected with only traces of kaempferol 3-glucuronide. This is in agreement with the results of Hillis [21] who found that myricetin is the major aglycone in the Occidentales series.

EXPERIMENTAL

Plant material. Leaf material was obtained from provenance trials planted at Alexandria University Experimental Station. 29 provenances from Alexandria, 5 local seed sources and 1 from the Negav. Local seeds were collected from: 1—Alexandria, 2—Mariot, 3—Modereat El-Tahrir, 4—Cairo, 5—Isna and 6—The Negav. The seedlings were raised in the nursery, and planted out when they were 4 months old. 3-Year-old trees were used for the present study. The site of the plantation has a heavy clay and slightly alkaline soil. Mature leaves were collected from 5 replicates for every provenance and combined.

Methods. The leaf tissue was extracted with 70% EtOH, dried and the extract fractionated using elution techniques. 2D-PC was applied for comparative studies. Chromatograms were examined under UV and sprayed with $AlCl_3$ and Benedict's reagent. Specific spray reagents used are: vanillin/HCl (procyanidins); $NaBH_4$/HCl (flavanones); Zn dust/HCl (3-hydroxyflavanones) and $FeCl_3$ (simple phenolics). Flavonoid glycosides were identified by standard methods [22, 23].

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