Antifungal substances found in leaves of Eucalyptus species[1]

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Summary. Antifungal substances were detected in leaves of 11 out of 27 Eucalyptus species examined. It was shown that antifungal substances found in the Eucalyptus species examined consist of from 1 to 4 different components.

In a previous paper[3] it was found that almost no microorganisms such as fungi, actinomycetes or bacteria were present inside or outside the leaves of Eucalyptus gunnii Hook, indicating the possible existence of antimicrobial substances in this species. Extraction of leaves of E. gunnii with several organic solvents and purification of these extracts on silica gel columns yielded 3 antifungal substances; gallic acid, an unidentified isomer of monoformyl-trihydroxy-benzene, and an unidentified isomer of diformyl-trihydroxy-benzene[4] which inhibited germination of conidia of Cochliobolus miyabeanus (S. Ito et Kuribayashi) Drechsler. This paper reports investigations of the antifungal substances present in an additional 27 Eucalyptus species.

Materials and methods. Leaves of 27 species of Eucalyptus were collected in South Australia. Each 1 g of air-dried leaves was soaked in 10 ml of methanol for about 7 days and then each extract was investigated for the presence or absence of antifungal substances. The microorganisms and assay methods in this study were Alternaria solani (Ell. et Mart.) Sor. and Cochliobolus miyabeanus (S. Ito et Kuribayashi) Drechsler for the conidial germination

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3 H. Egawa, I. Furusawa, S. Akai, K. Kobata, J. Fumoto, A. Kobayashi and K. Koshimizu, Proc. Kansai Plant Prot. Soc. *16*, 42 (1974).

4 A. Kobayashi, K. Koshimizu, T. Mitsui, H. Egawa and H. Fukami, Read at the Meeting of Japanese Agricultural Chemical Society, Tokyo (1972).

Table 1. Antifungal activity in methanol extracts of Eucalyptus species to various microorganisms

Eucalyptus species	Test microorganisms A. solani	C. miyabeanus	S. cerevisiae
E. albida Maiden & Blakely	–*	–	–
E. alpina Lindl	1**	1/4**	–
E. anceps R. Br. Blakely	–	–	–
E. angustissima F. Muell	–	–	–
E. apiculata Baker & Smith	–	1/2	–
E. brachyphylla C. A. Gardn	1	1/8	–
E. caesia Benth	–	–	–
E. concinna Maiden & Blakely	–	–	–
E. erythrandra Blakely & Steedman	–	–	–
E. erythrocorys F. Muell	–	1/2	–
E. erythronema Turcz	–	1/4	–
E. goniantha Turcz	–	–	–
E. grossa F. Muell	–	–	–
E. incurva Boomsa	–	1/4	–
E. kruseana F. Muell	–	1/4	13.9***
E. leptocalyx Blakely	–	–	–
E. macrandra F. Muell	–	–	–
E. merrickiae Maiden & Blakely	–	–	–
E. micrantha F. Muell	–	1/2	–
E. pachyphylla F. Muell	–	–	–
E. platypus Hook	–	1/4	–
E. preissiana Schauer	–	–	–
E. rhodantha Blakely & Steedman	–	–	10.0
E. spathulata Hook	–	–	–
E. torouata Leuhmann	–	–	–
E. triflora Blakely	–	1/2	–
E. websteriana Maiden	–	–	–

*, –, inactive. ** Maximum dilution of methanol extract which inhibited conidial germination of A. solani and C. miyabeanus. The original (undiluted) extract (1 g leaf/10 ml methanol) was taken as a concentration of 1. *** The diameter of inhibition zone (mm).

Table 2. Separation of the antifungal components in leaves of Eucalyptus species by thin layer chromatography

Eucalyptus species	Extraction solvents	Chromatographic solvents	No. of antifungal components	R_f-values
E. alpina	n-Hexane	B*	4	0.05(±**), 0.08(±), 0.22(+), 0.30(±)
	Benzene	B:EA=3:1(v/v)	3	0.64(+), 0.71(±), 0.78(±)
	Ethyl acetate	B:EA=1:1(v/v)	2	0.82(±), 0.87(+)
E. apiculata	Benzene	B:EA=3:1(v/v)	3	0.75(+), 0.82(+), 0.87(±)
E. brachyphylla	n-Hexane	B	2	0.00(+), 0.03(±)
	Benzene	EA	2	0.12(±), 0.18(±)
	Ethyl acetate	B:EA=1:1(v/v)	2	0.90(±), 0.93(±)
E. erythronema	Ethyl acetate	B:EA=1:4(v/v)	2	0.07(±), 0.14(+)
E. incurva	n-Hexane	B:n-H=5:1(v/v)	1	0.06(±)
	Benzene	B:EA=1:3(v/v)	1	0.04(±)
	Ethyl acetate	B:EA=1:5(v/v)	4	0.06(±), 0.10(+), 0.58(±), 0.65(+)
E. kruseana	n-Hexane	B	3	0.04(±), 0.28(+), 0.38(±)
	Benzene	B:EA=1:1(v/v)	2	0.89(+), 0.95(+)
E. gunnii	Benzene	EA	2	0.13(+), 0.20(+)
E. triflora	Ethyl acetate	B:EA=1:1(v/v)	1	0.07(±)

*B, Benzene; EA, ethyl acetate; n-H, n-hexane. **, Active; +, weakly active. Test organism: C. miyabeanus; test method: conidial germination.

test[3], and Saccharomyces cerevisiae Hansen for the paper disk method[3]. The level of antifungal activity was determined by preparing a 2fold dilution series of each methanol extract and assaying them for their inhibitory effect on conidial germination of the fungi and the growth of the yeast on paper disks.

Leaves of those species which contained antifungal activity in methanol extracts were then extracted sequentially with n-hexane, benzene, ethyl acetate and butanol. Extracts of each solvent were concentrated in a vacuum evaporator, suspended in deionized water and assayed for antifungal activity using the C. miyabeanus conidial germination test method.

Extracts of the above which showed antifungal activity were examined further by thin layer chromatography. R_f-values of the various components were measured and their antifungal activities were determined by testing methanol extracts of each component against C. miyabeanus.

Results and discussion. Antifungal activities detected in methanol extracts of the various species of Eucalyptus are shown in table 1. 2 species contained antifungal substances to A. solani, 10 species to C. miyabeanus, and 2 species to S. cerevisiae. 8 species contained antifungal substances which could be extracted by various solvents, indicating that there was probably more than 1 kind of component involved. These extracts (except those from the butanol and residual layers) were further separated by thin layer chromatography and found to consist of from 1 to 4 different components (table 2). At present, we do not know if these components are the same as or similar to those isolated from E. gunnii.

Myofibroblasts in hepatic schistosomal fibrosis[1]

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Summary. Myofibroblasts were identified in liver portal spaces of patients with Symmers' fibrosis following infection by Schistosoma mansoni.

In chronic human schistosomiasis the liver is always involved by intense fibrosis of multiple aspects. In severe and long-standing infections, classical Symmers' fibrosis develops. The principal characteristic of this lesion is scar-like fibrotic thickening of main portal spaces, some of which are joined with the Glisson's capsule forming deep furrows of sclerotic tissue at the surface of the liver[2]. Although this elementary lesion is one of the most frequent in tropical pathology, the cells implicated in its histogenesis are still little known.

Materials and methods. 32 surgical or percutaneous needle biopsies were taken from livers of patients chronically infected by Schistosoma mansoni. Patients were described in earlier publications[3,4]. Large portal spaces were identified macroscopically and fixed separately for this study. Small fragments of fresh tissue were fixed and treated by standard histological methods for optical microscopy. For electron microscopy, double glutaraldehyde/osmic fixation of simple osmic fixation were used, followed by dehydration in ethanol and propylene-oxyde and embedding in Epoxy resin. Semi-thin sections were treated by Richardson's technique, and examined for each fragment studied in electron microscopy.

Results. At a histological level, the main portal spaces were characterized by the following pathological modifications: granulomatous inflammatory reaction to schistosomal eggs, chronic endophlebitis and periphlebitis, progressive obliteration of portal vein with secondary angiomatous proliferation and progressive arterialization of portal liver circulation, moderate ductular proliferation, diffuse mononuclear and lymphoid infiltration and intense proliferation of connective tissue.

In the connective tissue, our attention was attracted to 3 morphologically similar cells. Classical fibroblasts were very frequent, embedded in dense collagen deposits, where they formed a loose and regular network. They were characterized by stellate form and elongated nuclei. In places where obliterated portal vein was located, large parallel bundles of cells were observed. They retained characteristics of smooth muscle cells, and they often had a circular distribution corresponding to the obliterated blood vessel. In certain regions of portal spaces, groups of elongated fusiform fibroblast-like cells were observed. They had morphological characteristics of classical fibroblasts but they could be distinguished by their regular fusiform shape and by their close cell-to-cell contacts, as they were tightly packed and arranged in long strands parallel to the main portal axis.

At the ultrastructural level, this last type of cells was identified as typical myofibroblasts[5] (figure 1). This identification was based on the following morphological characteristics, observed in portal spaces of all biopsies of schistosome-infected livers. Myofibroblasts were spindle-shaped with central elongated nucleus. The nuclei often showed indentations or folds, although this feature was less frequent than is classically described in scar tissues. Chromatin distribution in myofibroblasts in homogenous and dense chromatin patches was limited to a narrow zone underlying the nuclear membrane. Small nucleoli were often present. In the cytoplasm, rough endoplasmic reticulum and mitochondria were less developed than in normal fibroblasts. Golgi was always present and well developed (figure 1). A fibrillar system was always present in the cytoplasm, particularly in the regions subjacent to the cellular membrane (figure 2). The fibrils were arranged in groups, parallel to the elongated cell axis. Electron dense areas were scattered in the cytoplasm, most frequently at the periphery of the cell, immediately beneath the plasmalemma. At the same position, extremely numerous pinocytic vacuoles were observed, remarkably similar in size in all myofibroblasts, their diameter measuring 0.01–0.02 μm.

One of the most characteristic features of myofibroblasts was the presence of a well defined layer of material ressembling a basal membrane (figures 1 and 2). Often, this layer appeared as large tufts of fibrillar structures and