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The Isolation and Identification of Two Antifungal Pterocarpans from *Ulex Europaeus* L.

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ABSTRAK

Pengasingan dua sebatian pterokarpan daripada akar Ulex europaeus *(Leguminosae) dibincangkan. Sebatian tersebut dikenal pasti sebagai 3-hidroksi-8,9-metilenadioksipterokarpan (maackiain) dan 2-hidroksi-3, 4-dimetoksi-8, 9 metilenadioksipterokarpan [2-hidroksi-4-metoksipterokarpin) secara spektroskopi.*

ABSTRACT

The isolation of two antifungal pterocarpans from the roots of Ulex europaeus *L (Leguminosae) is described. The compounds were identified as 3-hydroxy-8,9-methylenedioxypterocarpan (maackiain) and 2-hydroxy-3,4-dimethoxy-8,9-methylenedioxypterocarpan (2-hydroxy-4-methoxypterocarpin) by spectroscopic methods.*

INTRODUCTION

Plants elicit antifungal compounds, phytoalexins, in response to challenge by fungal pathogens. Phytoalexins produced by plants of the family Leguminosae are often pterocarpans (e.g. compound (1), (2) and (3) (Pueppke and Van-Etten 1975)), although many other isoflavonoid compounds have been found to have antifungal activity (Russell *et al.* 1979; Sutherland *et al.* 1980, Lane *et al.* 1987). In the present paper, we wish to report the isolation and identification of maackiain (4) and the new pterocarpan (6) which show antifungal activity against *Cladosporium cladosporoides* in a TLC plate bioassay. These compounds were isolated from the roots of *Ulex europaeus* L. (Leguminosae), an exotic shrub found throughout New Zealand.

METHODS AND MATERIALS

Antifungal Assays.

Antifungal activity in crude fractions was monitored by fungal spore germination assays on TLC plates. Eluted plates were sprayed with a suspension of conidia of the pigmented fungus *Cladosporium cladosporioides* in a nutrient solution (Bailey and Burder 1973) and examined after an incubation period of three days at room temperature in a water saturated atmosphere. Antifungal activity showed as inhibition zones.

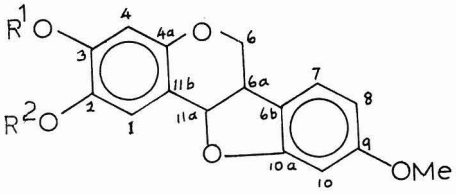
Extraction of Roots.

Dried root of *Ulex europaeus* L. (2.35kg) was extracted with MeOH in a Soxhlet apparatus for 48 hr and the solvent was evaporated under reduced pressure to give crude product (450 g). The crude extract (20 g) was fractionated using flash chromatography on reversed-phase silica (Kuhler and Lindsten 1983) and elution with methanol-water mixtures.

The fractions were spotted on T.L.C. and tested for antifungal activity (Table 1).

Isolation of Pterocarpans.

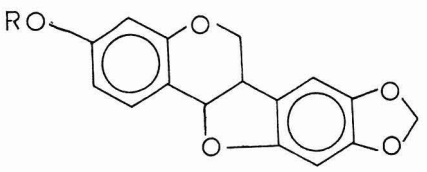
The active fraction, (H_2O-MeOH (1:4) (3.6g)) was flash-chromatographed on silica gel (30 g) with dichloromethane-methanol mixtures. Elution with dichloromethane gave a fraction



(1) R^1 = Me , R^2 = H

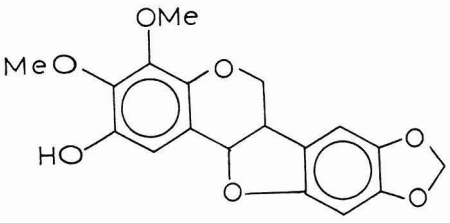
(2) R^1 = R^2 = Me

(3) R^1 = Me , R^2 = Ac

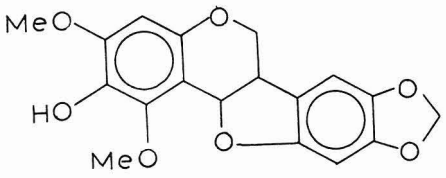


(4) R = H

(5) R = Me



(6)



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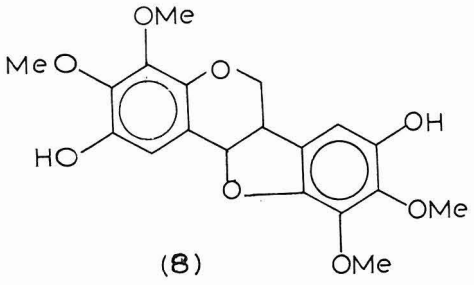

(8)

TABLE 1
Fractionation of the crude extract

NUMBER	ELUENT	ANTIFUNGAL ACTIVITY
1.	H_2O	–
2.	H_2O-MeOH (3:1)	–
3.	H_2O-MeOH (3:2)	–
4.	H_2O-MeOH (1:1)	–
5.	H_2O-MeOH (2:3)	–
6.	H_2O-MeOH (1:4)	+
7.	H_2O-MeOH (1:9)	–
8.	MeOH	–
9.	MeOH-CH_2Cl_2 (3:1)	–
10.	MeOH-CH_2Cl_2 (1:1)	–
11.	CH_2Cl_2	–
12.	MeOH	–

containing the pterocarpans (255 mg) and was re-chromatographed on Sephadex LH20 (100 g). Elution with dichloromethane and further purification using HPLC (ODS, MeOH-H_2O, 3:2) gave the pterocarpan (6) (15 mg). Elution of the Sephadex LH20 with dichloromethane-acetone (3:1) gave maackiain (4) (30 mg).

6a,11a-2-Hydroxy-3,4-dimethoxy-8,9-methylenedioxypterocarpan (2-hydroxy-4-methoxypterocarpin) (6). Mp 120-21° υmax 3400 (OH), 1500, 1600, 1620 (aryl) cm^{-1}; λmax (MeOH) 230 (sh) (log ε 3.60), 303 nm (log ε 3.80). M found: 344.0904; $C_{18}H_{18}O_7$ requires: 344.0894. ^1H NMR (80 MHz, $CDCl_3$) δ: 3.4-3.7 (m, H-6ax, H-6a), 3.87 (s, OMe), 3.94 (s, OMe), 4.26 (dd, J=3.6, 10 Hz, H-6 eq), 5.41 (s, OH), 5.41 (d, J=6.9 Hz, H-11a), 5.87 (d, J=1.3 Hz) and 5.90 (d, J=1.3 Hz)

(-OCH_2 O-), 6.40 (s, H-10), 6.69 (s, H-7) 6.82 (s,H-1). M/z: 344 (47), 329 (10), 314 (10), 167 (7), 162 (6).

The acetate prepared with acetic anhydride-pyridine (1:2, 50°, 4 hr) crystallised from aqueous acetone, mp 133°. ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.28 (s, MeCOO), 3.3-3.37 (m, H-6ax, H-6a), 3.86 (s, 2xOMe), 4.27 (m, H-6eq), 5.41 (d, J=6Hz, H-11a), 5.88 (br.s, OCH_2O), 6.39 (s, H-10), 6.69 (s, H-7), 6.95 (s, H-1).

6a,11a-3-hydroxy-8,9-methylenedioxypterocarpan (maackiain) (4)

UV, EI-MS and ^{1}H NMR data were in agreement with reported values (Briggs *et al.* 1975). T.L.C. was consistent with an authentic sample obtained from previous work (Sutherland *et al.* 1980).

RESULTS AND DISCUSSION

The root extractives of *Ulex europaeus* yielded two pterocarpans. The structures were elucidated by spectroscopic methods. The major constituent, $C_{16}H_{12}O_5$, was the known compound maackiain (4) and the minor component, $C_{18}H_{16}O_7$, was the new 2-hydroxy-4-methoxypterocarpin (6).

The ^{1}H NMR spectrum of both compounds showed the presence of a methylenedioxy moiety (δ 5.85) and the characteristic ABMX pattern for the 6, 6a and 11a protons of the fused pyranofuran rings of pterocarpans (multiplet δ 3.5, double doublet δ 4.26, doublet δ 5.41). The aromatic protons for ring B (H-7, H-10) in both compounds were seen as two singlets at δ 6.40 and 6.69 confirming the presence of the 8,9-aryl methylenedioxy moiety in 6 (Donelly and Fitzgerald 1971). The presence in 6, of two methoxyl groups (δ 3.87 and δ 3.94) and one further aromatic proton singlet at δ 6.82 suggesting that ring A is trisubstituted is consistent with that reported for compound 8 (Filho *et al.* 1980). The proton at C-1 in 8 occurs at δ 6.90 while the proton at C-4 in 7 occurs at δ 6.30. That ring A of 6 and 8 were similarly substituted, was confirmed by ^{1}H NMR analysis of the mono-acetate and of the paramagnetic pyridine-induced proton shift. The acetate-induced shift of H-1 in compound 8 is Δ0.15 and of H-4 in compound 7 is Δ0.08 (Filho *et al.* 1980). Compound 6 on acetylation gave a signal at Δ0.13 consistent with a C-1 proton adjacent to a hydroxyl group. In pyridine, 6, gave H-1 at Δ0.40 consistent with that reported by Filho *et al.* 1980 (Δ0.49 for H-1, Δ0.27 for H-4).

Confirming evidence for structure 6 was provided by the ^{13}C NMR data (Table 2). Resonances identical to those of the B and C ring carbons for pterocarpin (5) (Pelter *et al.* 1976) were observed. Using data for a number of Ring A substituted isoflavones (Murphy *et al.* 1986) and calculating the expected resonances for a subsititute pterocarpan, it was found that the data was consistent with 2,3,4-oxy substitution rather than 1,2, 3- or 1,3,4-substitution patterns.

TABLE 2.
C13 NMR of pterocarpans

Compounds	5*	6	6 calc.
C-11b	112.3	116.0	113.4
C1	131.6	109.9	105.9
C-2	109.0	140.8	136.7
C-3	160.9	142.9	142.1
C-4	101.5	141.3	137.7
C4a	156.4	143.6	142.3
C6	66.4	66.8	
C-6a	40.2	40.5	
C-11a	78.4	78.3	
C-6b	117.8	117.7	
C-7	104.6	104.6	
C-8	141.5	141.8	
C-9	147.9	148.2	
C-10	93.7	93.7	
C-10a	154.1	154.1	
O-CH_2-O	101.1	101.2	
OMe	55.26	61.2	
OMe		61.1	

* Data from Pelter *et al.*, 1976.

Both maackiain and 2-hydroxy-4-methoxypterocarpin (6) showed similar levels of antifungal activity in the TLC plate bioassay.

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