Pergamon

0031-9422(94)00661-X

Phytochemistry, Vol. 38, No. 4, pp. 1033–1035, 1995

0031-9422/95 $9.50 + 0.00

AN ANTIMICROBIAL KAEMPFEROL-DIACYL-RHAMNOSIDE FROM *PENTACHONDRA PUMILA*

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(*Received* 20 *June* 1994)

Key Word Index—*Pentachondra pumila*; Epacridaceae; antimicrobial; flavonol; kaempferol 3-(2,4-di-*E*-*p*-coumaroylrhamnoside).

Abstract—An extract of *Pentachondra pumila* yielded a new compound, kaempferol 3-(2,4-di-*E*-*p*-coumaroylrhamnoside) as the antimicrobially active component. Analysis of 13 other extracts of various New Zealand Epacridaceae species revealed this compound to be a constituent of all but one.

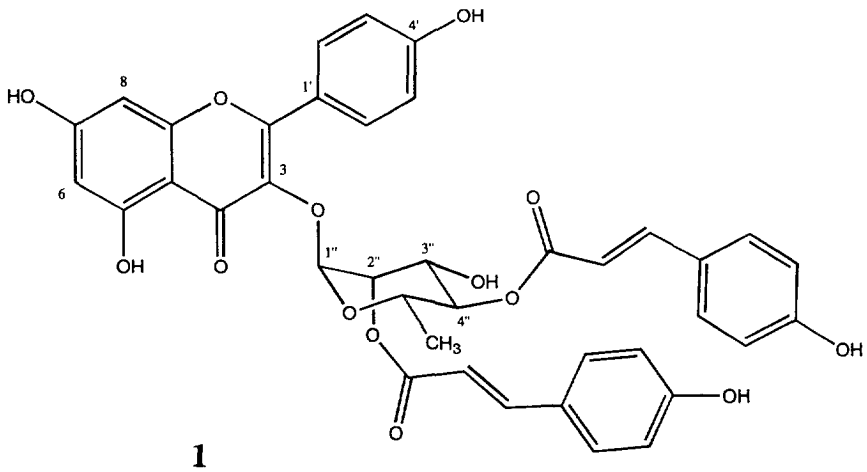
INTRODUCTION

During an investigation of New Zealand plant extracts as sources of bioactive compounds, it was observed that several species of the Epacridaceae family showed inhibitory activity against multiresistant *Staphylococcus aureus* (MRSA). The relatively high proportion of species showing anti-MRSA activity (Table 1) indicated this family to be worthy of detailed investigation as to the source of this activity. This family has a mainly Australasian distribu-

Table 1. Antimicrobial activity and occurrence of **1** in some Epacridaceae species

Plant species	Herbarium no. (CHR)	Inhibition of multiresistant MRSA*	Relative amount of **1**†
Subfamily Epacrideae			
Archeria traversii (Hook. f.)	467 778	–	0.46
Dracophyllum acerosum (Bergg)	439 768	–	0.35
D. latifolium (A. Cunn.)	472 981	+	0.41
D. lessonianum (A. Rich)	472 953	+	0.98
D. longifolium var. (J. R. et G. Forst.) R. Br.	465 802	–	0.00
D. sinclairii (Cheesem.)	472 983	±	0.63
D. uniflorum (Hook. f.)	465 816	–	0.18
Epacris alpina (Hook. f.)	467 757	–	0.32
E. pauciflora (A. Rich.)	472 954	+	0.68
Subfamily Styphelieae			
Cyathodes empetrifolia (Hook. f.)	467 755	–	0.46
Leucopogon fasciculatus (A. Rich.)	461 089	–	0.33
L. fraserii (A. Cunn.)	467 761	–	0.58
L. suavolens (Hook. f.)	467 751	±	0.64
Pentachondra pumila (J. R. et G. Forst.) R. Br.	465 820	+	1.00

*See Experimental for details.
†*Pentachondra pumila* arbitrarily assigned as 1.00.



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tion with the major New Zealand genus being *Dracophyllum*. From amongst those extracts showing bioactivity, *Pentachondra pumila* (J. R. et., G. Forst.) R. Br. was selected for initial investigation. Bioassay-guided fractionation of extracts from this small low-growing shrub led to the isolation of an unusual non-polar flavonol glycoside as the active component.

RESULTS AND DISCUSSION

A methanolic extract of dried leaf material was chromatographed by silica gel column chromatography, Sephadex LH-20 column chromatography and finally reversed phase HPLC to yield **1** as the major antimicrobial compound.

From preliminary NMR spectra, **1** was clearly a kaempferol 3-rhamnoside and closer examination of ^{13}C NMR and FAB-MS data ([MH]$^+$ 725 amu) gave a molecular formula of $C_{39}H_{32}O_{14}$, consistent with the rhamnosyl group substituted at two sites with *p*-coumaroyl groups. A H–H COSY experiment showed clear coupling connections around the rhamnopyranoside ring and proved the protons at the acyl-substituted positions, i.e. those shifted downfield from methylrhamnoside, to be those at C-2 (5.52 ppm) and C-4 (4.90 ppm). This substitution pattern assignment was supported by comparison of ^{13}C NMR data of **1** with those previously reported for kaempferol 3-(2,3-di-*E*-*p*-coumaroylrhamnopyranoside), **2** (Table 2) [1]. Also comparison with relevant ^{13}C NMR data for kaempferol 3-rhamnoside (Table 2) showed expected upfield shifts for C-1, -3 and -5, i.e. those adjacent to acylation sites. The *E*-configurations of the *p*-coumaroyl groups and the α-linkage of the rhamnose were assigned from the ^{1}H NMR coupling constants (16 and 1.6 Hz, respectively). Thus, **1** is a new compound, kaempferol 3-(2,4-di-*E*-*p*-coumaroyl-α-L-rhamnopyranoside) [2], and is one of the few examples of a diacylated kaempferol rhamnoside.

Since several other Epacridaceae species also showed anti-MRSA activity, all of the available extracts from this family were examined for the presence of **1**. Small portions of previously prepared, freeze-dried extracts, were suspended in methanol and a sample of each analysed by HPLC. The relative amount of **1** in each extract (Table 1) appears to correlate reasonably well with the observed anti-MRSA activity. With the exception of *D. longifolium*, **1** was present in all of the species examined. The Epacridaceae family has been the subject of several previous studies concerning the occurrence of anthocyanins [3] and flavonol arabinosides [4]. While only a small number of species was examined in the present study, the results do suggest that compounds such as **1** are widespread in this family and may serve as useful chemotaxonomic markers.

Table 2. ^{13}C NMR data for **1**, **2** and kaempferol 3-rhamnoside

C atom	**1** (Acetone-d_6)	**2** (Methanol-d_4)*	K-3-rhamn (Methanol-d_4)*
2	158.2	158.8	
3	134.1	135.2	
4	179.0	178.9	
5	163.0	163.0	
6	99.7	100.2	
7	166.4	165.8	
8	94.8	95.7	
9	158.8	158.8	
10	105.8	104.8	
1′	122.4	122.5	
2′,6′	131.9	131.8	
3′,5′	116.5	116.9†	
4′	158.8	161.5	
1″	99.1	101.2	103.5
2″	72.5	72.2	72.2
3″	68.2	73.1	72.0†
4″	74.4	71.0‡	73.3
5″	69.4	70.9‡	71.9†
6″	17.8	17.8	17.6
1 coum	127.9, 127.0	127.0, 126.9	
2,6 coum	131.2, 131.1	131.4, 131.2	
3,5 coum	116.7, 116.7	117.0,† 116.8†	
4 coum	160.8, 161.2	161.8, 161.8	
7 coum	146.5, 145.8	147.8, 147.0	
8 coum	115.5, 115.2	114.8, 114.2	
9 coum	166.7, 167.1	168.5, 167.8	

*Data taken from ref. [1].
†‡Interchangeable within column.

EXPERIMENTAL

General. Samples of plant material were collected from various localities in New Zealand and voucher specimens have been deposited in the Landcare NZ Herbarium, Christchurch (CHR #, Table 1). Leaf and stem material from each plant was air-dried, shredded and extracted by soaking in aq. EtOH. After defatting with hexane the extract was freeze-dried and stored at −20°. NMR spectra; Bruker AC300. Mass spectra; VG 70-250S. HPLC analyses were performed using a Waters 600 system with diode array detection.

Isolation of **1**. Frozen plant material of *P. pumila* (75 g) collected near Arthurs Pass, NZ, (CHR 471368) was macerated and extracted by soaking in MeOH. This extract (*ca* 8 g) was then subjected to vacuum CC on SiO_2 eluting with a hexane → EtOAc gradient. The active fr. (*ca* 300 mg) was further purified by LH-20 (MeOH) and prep. RP HPLC [MeCN:5% aq. HCO_2H (1:1)]. Compound **1** was obtained as a yellow powder (6 mg). UV λ_{max}^{MeOH} nm: 270, 300 (sh), 316. FAB-MS 725 $[MH]^+$, 439 [65, rha + coum(×2)], 147 (100, coum); ^1H NMR (acetone-d_6): δ 12.5 (1H, *s*, 6-OH), 7.82 (2H, *d*, J = 8.7 Hz, H-2′, -6′), 7.58 (1H, *d*, J = 16 Hz, 7-coum), 7.49 (4H, *d*, J = 8 Hz, 2,6-coum), 7.47 (1H, *d*, J = 16 Hz, 7-coum), 7.04 (2H, *d*, J = 8.7 Hz, H-3′, -5′), 6.81 and 6.80 (2H each, *d*, J = 8.6 Hz, 3,5-coum), 6.38 (1H, *d*, J = 1.6 Hz, H-8), 7.20 and 6.34 (1H each, *d*, J = 16 Hz, 8-coum), 6.18 (1H, *s*, H-6), 4.72 (1H, *d*, J = 1.4, H-1″), 5.52 (1H, *m*(*dd*), H-2″), 4.90 (1H, *t*, J = 9.8 Hz, H-4″), 4.08 (1H, *m*, H-3″), 3.31 (1H, *m*, H-5″), 0.77 (3H, *d*, J = 6.2 Hz, H-6″). ^{13}C NMR: Table 2.

Analysis of crude extracts of Epacridaceae species. A portion (50 mg) of each extract was soaked in 0.5 ml of MeOH for 16 hr. The filtered solns were then analysed by RP HPLC. The peak corresponding to **1** was eluted at *ca* 19 min using a gradient elution system of 5% aq. HCO_2H in MeCN (80:20 → 0:100). Relative amounts of **1** in each extract were determined from peak height.

Antimicrobial testing. These were performed by Environmental Science Research Institute, Porirua, NZ. Test plates were prepared from agar + extract to give a final concn of 100 μg extract ml^{-1} agar. Plates inoculated with multiresistant *S. aureus* (MRSA) [strain SK18] were incubated overnight and plates scored as no inhibition (−), some reduction in growth (±) or no growth (+).

Acknowledgements—The author thanks A. P. Druce (Landcare NZ) and W. Nelson (Museum of New Zealand) for their assistance in the collection and identification of plant material.

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