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Simple 1,4-benzoquinones with antibacterial activity from stems and leaves of *Gunnera perpensa*

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Abstract

From the dichloromethane extract of the leaves and stems of *Gunnera perpensa* two new, simple 1,4-benzoquinones and a known benzopyran-6-ol were isolated. From the methanol extract phytol was obtained. The two benzoquinones, 2-methyl-6-(-3-methyl-2-butenyl)benzo-1,4-quinone (**1**) and 3-hydroxy-2-methyl-5-(3-methyl-2-butenyl)benzo-1,4-quinone (**2**) and the benzopyran, 6-hydroxy-8-methyl-2,2-dimethyl-2H-benzopyran (**3**) were examined for antimicrobial properties together with the crude stem, leaf and root extracts. Minimum inhibitory concentration (MIC) assays were used to quantify antimicrobial activity and the MIC values for the crude extracts of stems, roots and leaves ranged between 100 μg and >16 mg/ml against the eight microorganisms investigated. Compound **1** showed significant antimicrobial activity with the most sensitive organism being *Staphylococcus epidermidis* with an MIC of 9.8 μg/ml. For compound **2**, no activity was noted. Compound **3** exhibited good activity against the yeasts *Cryptococcus neoformans* (75 μg/ml) and *Candida albicans* (37.5 μg/ml).

Keywords: Prenylated 1,4-benzoquinones; *Gunnera perpensa* leaves, roots and stems; Antimicrobial

1. Introduction

We have recently reported (Khan et al., 2004) on the active compounds in the roots of *Gunnera perpensa* (*Haloragaceae*) and were able to show that the phenylpropanoid, *Z*-venusol, effects contraction of uterine smooth muscle. This is in keeping with the traditional use of the roots of the plant to aid in *post-partum* expulsion of the retained placenta in cattle (Hutchings et al., 1996). Other uses of root decoctions of the plant are for relief of menstrual pains and also as a cure for female infertility (Van Wyk et al., 1997).

The older literature says little about the specific use of the leaves and stems of *Gunnera perpensa*, although the large rounded leaves are responsible for its common name "river pumpkin". There is, however, mention in a paper of Bryant (1909) that the leaves are employed by the Zulus as an emetic and Phillips (1917) records that the stems can be eaten when fresh. Hutchings et al. (1996) makes brief reference to the traditional use of the roots and leaves for the treatment of psoriasis.

Our interest in the aerial parts of the plant arose when we noticed that the fresh stems had a distinct red colour and that contact with the leaves caused slight skin irritation. In addition, our scanning of the recent literature on antibacterial substances revealed that the leaves of *Gunnera perpensa* are used

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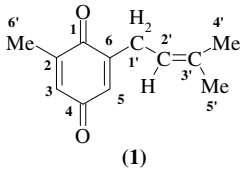
by the rural inhabitants of the Eastern Cape Province as a dressing for wounds (Grierson and Afolayan, 1999).

2. Results and discussion

Extraction of the stems and leaves followed by initial column chromatographic separation and final purification by radial chromatography afforded two 1,4-benzoquinone derivatives (**1**) and (**2**). These were identified as 2-methyl-6-(3-methyl-2-butenyl)benzo-1,4-quinone and 3-hydroxy-2-methyl-5-(3-methyl-2-butenyl)benzo-1,4-quinone, respectively. A third compound proved to be 6-hydroxy-8-methyl-2,2-dimethyl-2H-benzopyran (**3**).

From the methanolic extract of the aerial parts of the plant the known compound *trans*-phyt-2-enol (**4**) (Sims and Pettus, 1976; Aoki et al., 1982; Brown, 1994) was obtained. Phytol is a mild skin irritant obtained from nettles and numerous other plants (Lewis, 1992), and it was probably the reason for the observed skin irritation during handling of the plant.



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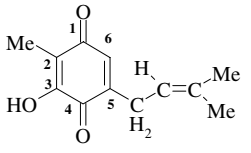

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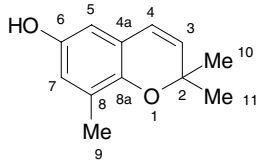

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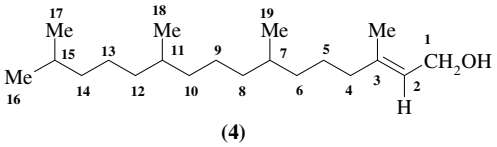

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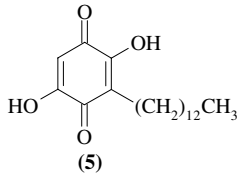

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Two aspects of the present work we found intriguing:

(i) While 1,4-benzoquinones are common in Nature (Thomson, 1971) and while many possess complex side chains, as, for example in rapanone (**5**), or 6,6′-biembelin (**6**), we were surprised that the existence of the structurally simple compounds **1** and **2**, has not been reported previously. A structural isomer of **1** has, however, been isolated from *Pyrolia media* (*Pyrolaceae*) (Burnett and Thomson, 1968).

(ii) Antimicrobial assessment of the crude extracts (Table 1) indicates that the highest sensitivity was obtained from the leaf extracts followed by the stems, with the least activity noted for the root extracts. The results from the antimicrobial tests on compounds **1**, **2** and **3** emphasized again the effect of small structural changes on overall activity. Compound **1** afforded very positive results. Minimum inhibitory concentration assays (MICs) on **2** (not shown in the table) indicated no activity against any of the pathogens. Rather surprisingly the presence of the hydroxyl group in **2** did not promote activity. For **3** some significant antimicrobial activity was observed especially for *Candida albicans* (37.5 μg/ml) with lower sensitivities (75–750 μg/ml) against the other seven test organisms.

2.1. Characterisation of compounds

Initially it was a problem to establish the class of natural products to which our compounds belonged. This was complicated by the fact that the ^{13}C spectrum of **2** indicated two carbonyl compounds (183 and 187 ppm) whereas **1** which was obviously closely related, had a single carbonyl at 188 ppm. Once it was realised that this single resonance represented two overlapping carbonyl groups, the structural data fell into place.

Using HMQC analysis it was clear that both compounds possessed the prenyl, (3-methyl-2-butenyl), side chain and it was a matter of allocating the relative positions of the remaining substituents (Table 2). For **1** the H-5 and H-3 protons (δ 6.42, 6.50, respectively) on either side of the carbonyl group at C-4, could be assigned readily. The methylene group on the prenyl side chain indicated strong correlations to H-5, C-6 and C-1 so that the point of attachment of the prenyl group could be established. Thereafter, the HMQC correlations of the ring methyl group to C-2, as well as to H-3 and C-1 confirmed its attachment to C-2. Final proof of structure came from the high resolution mass spectrum *m/z* 190.09867 M^+ calc. For $C_{12}H_{14}O_2$ = 190.09938.

In compound **2** the two conjugated carbonyls were clearly discernable at 183 and 187 ppm, in common with

Table 1
MIC values (μg/ml) for compounds **1** and **3** and *Gunnera perpensa* aq. root, stem and leaf extracts

Pathogen	Control MIC (μg/ml)	Compound **1** MIC (μg/ml)	Compound **3** MIC (μg/ml)	Aq. root extract MIC (μg/ml)
Escherichia coli (ATCC 11775)	0.63	>6250	750	4000
Klebsiella pneumoniae (NCTC 9633)	0.20	>6250	187	6400
Enterococcus faecalis (ATCC 29212)	6.25	39	375	8000
Staphylococcus aureus (ATCC 6538)	0.31	39	131	4000
Bacillus cereus (ATCC 11778)	2.5	18	75	4000
Staphylococcus epidermidis (ATCC 2223)	1.25	9.8	187	6000
Cryptococcus neoformans (ATCC 90112)	2.5	70	75	750
Candida albicans (ATCC 10231)	1.25	130	37	4000

Tests were done at least in duplicate and ciproflaxin (for bacteria) and amphotericin B (for yeasts) were used as controls.

Table 2
^{1}H and ^{13}C data for compounds **1** and **2**

Atom no.	Compound **1**		Compound **2**	
	^{1}H NMR (*J* in Hz)	^{13}C NMR	^{1}H NMR (*J* in Hz)	^{13}C NMR
1	–	187.8	–	187.7
2	–	145.7	–	117.4
3	6.50 (1H, *s*)	132.9	–	151.7
4	–	187.8	–	183.2
5	6.42 (1H, *s*)	132.5	–	150.9
6	–	148.3	6.46 (1H, *s*)	127.4
1′	3.07 (2H, *d*, *J* = 7.5)	27.5	3.15 (2H, *d*, *J* = 6.8)	28.1
2′	5.10 (1H, *bt*, *J* = 7.5)	118.1	5.13 (1H, *bt*, *J* = 7.5)	118.0
3′	–	136.0	–	136.4
4′	1.69 (3H, *s*)	25.6	1.62 (3H, *s*)	25.7
5′	1.58 (3H, *s*)	17.5	1.75 (3H, *s*)	17.7
6′	2.00 (3H, *s*)	15.8	1.94 (3H, *s*)	8.1

many substituted 1,4-benzoquinones (Manguro et al., 2003). The prenyl side chain was identified as for compound **1**. In this instance, there was only one sp^2 proton on the ring (δ 6.46 for H-6). It had strong correlations in the HMQC spectrum to C-1, C-5 and the CH_2 of the prenyl group. This left the hydroxyl and methyl groups on the ring at C-3 and C-2, respectively, or with the positions inverted. HMQC correlations indicated strong connectivities of OH to C-3, C-4 and C-2 while the methyl group had cross-peaks to C-1, C-2 and C-3, thus verifying the allocations shown in structure **2**. These assignments were also supported by the IR absorption. In compound **1** (in which no H-bonding is possible to C-4) the carbonyl peak was at 1656.23 cm^{-1}. Similarly in **6** (no H-bonding) the peak was at 1659.0 cm^{-1} (Burnett and Thomson, 1968). As can be anticipated H-bonding to C-4 in **2** lowers the carbonyl absorption to 1645.1 cm^{-1}. High resolution mass spectrometry results were in complete accord with the assigned allocations, *m/z* 206.09619, M^+ calc. For $C_{12}H_{14}O_3$ = 206.09429.

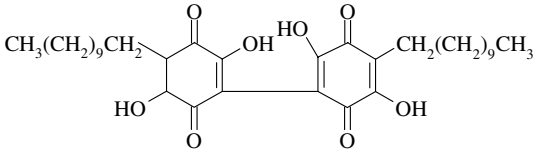
Compound **3** has been isolated once previously from *Pteris ryukyuensis* (*Pteridaceae*) by Tanaka et al. (1978) and given the name Pterochromene L1. The spectral data provided leave no doubt that **3** is identical. Since proton spectra were recorded at 60 MHz and no carbon values were given, the spectral values are recorded here as follows: ^{1} NMR ($CDCl_3$, 500 MHz), 6.47 (1H, *d*, *J* = 2.6 Hz, H-7). 6.32 (1H, *d*, *J* = 2.6 Hz, H-5), 6.22 (1H, *d*, *J* = 9.6 Hz, H-4), 5.61 (1H, *d*, *J* = 9.6 Hz, H-3), 2.13(3H, *s*, 8-Me) 1.39 (6H, *s*, 2,2-diMe); ^{13}C NMR ($CDCl_3$, 125 MHz), 148.9 (C-6), 145.0 (8a), 131.9 (C-3), 126.9 (C-8), 122.7 (C-4), 121.8 (C-4a), 117.2 (C-7), 110.4 (C-5), 75.7 (C-2), 27.8 (C-10, 11), 15.7 (C 9). EIMS *m/z* (rel. int.): 190 (40), 176 (22), 175 (100), 91 (6), 77 (5). HRMS *m/z* 190.10064 (M^+) (calcd. for $C_{12}H_{14}O_2$ 190.09938).

2.2. Antimicrobial tests

The results for compounds **1** and **3** and the aqueous extracts of *Gunnera perpensa* roots, stems and leaves are shown in Table 1.

Compound **1** shows excellent inhibition of *Staphylococcus epidermidis* and the MIC values for *Enterococcus faecalis, Staphylococcus aureus* and *Bacillus cereus* are promising. These values are significant when considering the views expressed on anti-staphylococcal plant natural products (Gibbons, 2004). In this review paper, only pure compounds with MIC values of less than 64 μg/ml were selected by the reviewer. An example of a compound obtained from the roots of a plant and used as a treatment for

gastrointestinal disorders in Mexico is the compound beyerenoic acid (**7**) (Zamilpa et al., 2002). It has a MIC value towards *Staphylococcus aureus* and *Enterococcus faecalis* of 12 μg/ml. In relation to the observed activity of **1** it is interesting to note that a publication by Donowitz et al. (1982) points out that patients admitted as Intensive Care Unit patients have a higher risk of nosocomial infections than other hospitalized patients and that the most common bloodstream infections were *S. epidermidis* and *S. aureus*. Infections from these opportunistic pathogens could possibly be avoided with traditional use of *Gunnera perpensa.* Activity for the leaves is higher in all cases compared with the stems, thus justifying the practice of using leaves to prepare wound dressings. While the activities recorded for the leaf extract is not impressive, the inhibition of *S. aureus* (MIC 100 μg/ml) and *S. epidermidis* (MIC 120 μg/ml) are good and probably reflect the effect of compound **1** in this part of the plant.



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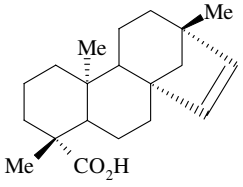

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3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian 500 MHz spectrometer. High resolution masses were from a Kratos MS 80 RF double-focussing magnetic sector instrument at 70 eV.

3.2. Plant material

Gunnera perpensa leaves and stems were collected in October 2003 on a farm in the Underberg district of KwaZulu-Natal. A voucher specimen (leaves, stems and root) was deposited in the Bews Herbarium, University of KwaZulu-Natal Pietermaritzburg (lodged under S.E. Drewes, No. 8). Verification was by the curator of the herbarium, Prof. T. Edwards.

3.3. Extraction and isolation

The fresh stems (279 g) and leaves (150 g) were extracted at room temperature for two days. Preliminary purification by column chromatography (Si gel) using $CHCl_3$ as eluent afforded a brown residue (195 mg) from the stems which was further purified by centrifugal chromatography $CHCl_3$ to afford 7 mg of oil [compound **1**] and orange crystals (25 mg, m.p. 78–79 °C). A similar separation and purification of the leaves gave pure compound **1** [52 mg, orange oil] and orange crystals (15 mg) of **2**. Further purification of the fractions by CH_2Cl_2 yielded **3** (36.3 mg from the stems and 27.4 mg from the leaves). In chloroform, the R_f's of **1**, **2** and **3** on a TLC plate were 0.77 and 0.57 and 0.23, respectively. Spectral data for **1** and **2** are shown in Table 2.

The combined plant material from the leaves and stems was subsequently extracted with MeOH (8.03 g). Column chromatography (Si gel) with MeOH–$CHCl_3$ (3:17) afforded one major high R_f fraction and two minor ones at lower R_f. A second column chromatographic separation using $CHCl_3$ gave phyt-2-enol (15 mg). Comparison of spectral data from Sims and Pettus (1976), Aoki et al. (1982) and Brown (1994) confirmed the identity of the compound as *trans*-phyt-2-enol (**4**).

3.4. Antimicrobial determination

Minimum inhibitory concentrations were determined using the INT microplate method as described by Carson et al. (1995) and Eloff (1998). Pathogens tested and corresponding ATCC numbers are given in Table 1. The plant extract and compounds were diluted twofold in each successive serial dilution. Cultures were grown overnight at 37 °C for 24 h, diluted 1:100 and inoculated into all wells and incubated for 37 °C for 24 h for bacteria and 48 h for yeasts. A 0.2 mg/ml *p*-iodonitrotetrazolium violet (INT) solution was transferred to all inoculated wells and examined to determine a colour change in relation to concentration of microbial growth after 6 h for bacteria and 24 h for yeasts.

Acknowledgements

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