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THE GLYCOSIDIC PRECURSOR OF (*Z*)-5-ETHYLIDENE-2(5*H*)-FURANONE IN *HALOCARPUS BIFORMIS* JUVENILE FOLIAGE

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Key Word Index—*Halocarpus biformis*; *H. bidwillii*; *H. kirkii*; Podocarpaceae; juvenile foliage; glycosides; lactones; (*Z*)-5-ethylidene-2(5*H*)-furanone.

Abstract—A new glycosidic lactone, (5*R*,6*R*)-5-(1-hydroxyethyl)-2(5*H*)-furanone β-D-glucopyranoside, has been identified as the principal precursor of (*Z*)-5-ethylidene-2(5*H*)-furanone in juvenile foliage of the New Zealand tree *Halocarpus biformis*. Three related lactone glycosides were isolated in smaller amounts, together with the known phenolic glycosides pyroside, arbutin and picein. The principal lactone glycoside underwent facile elimination of glucose, in neutral or basic conditions, to yield (*Z*)-5-ethylidene-2(5*H*)-furanone and its *E*-isomer. This lactone glycoside was also detected in foliage of *H. bidwillii* and *H. kirkii*.

INTRODUCTION

New Zealand trees and shrubs in the Podocarpaceae have proved a rich source of terpenes, particularly diterpenes [1, 2]. A wide range of flavonoids have also been identified from these plants [2]. However, there do not seem to have been any investigations on H_2O-soluble compounds in the foliage of New Zealand podocarps. We now report the isolation of a new glycosidic lactone, which is the principal precursor of (*Z*)- and (*E*)-5-ethylidene-2(5*H*)-furanones, **1** and **2**, from the juvenile foliage of the small tree *Halocarpus biformis* (Hook.) Quinn. Several other glycosides were also found, both new and known.

The starting point for this research came during our investigation of the terpenes obtained by hydro-distillation of foliage of *H. biformis* [previously named *Dacrydium biforme* (Hook.) Pilger] [3]. The juvenile and adult foliage of *Halocarpus* species are very different: the flat, linear juvenile foliage contrasts strongly with the adult foliage of scale-like leaves, closely appressed to branchlets [4]. In fact *H. biformis*, which is only found in New Zealand, is named after these two forms of foliage. The genus contains only two other species, *H. kirkii* (Parl.) Quinn and *H. bidwillii* (Kirk) Quinn, which are also endemic to New Zealand [5, 6].

We found that distillates of juvenile foliage of *H. biformis* contained (*Z*)-5-ethylidene-2(5*H*)-furanone (**1**), but this compound was not detected in distillates of adult foliage [3]. We noted then that the lactone **1** was an artefact produced by the distillation [3]. We did not detect **1** in our subsequent work on hydro-distillates from juvenile and adult foliage of *H. bidwillii* [7].

RESULTS AND DISCUSSION

Lactone (**1**) *as an artefact*

As lactone **1** was obtained from *H. biformis* juvenile foliage by hydro-distillation with concurrent extraction into hexane [3], but was not present in a cold ethyl ether extract, it seemed that **1** was formed in the extraction process. The closely related compound, 5-methylene-2(5*H*)-furanone (protoanemonin) (**3**), is not present in intact plants, but is released enzymatically from the lactone glycoside ranunculin (**4**) when plant tissues are ground [8]. Although ground *H. biformis* foliage turned red-brown in under an hour and developed a different aroma (intact foliage remained green for days), this change was not accompanied by release of lactone **1**. Therefore, **1** seemed to be an artefact of hydro-distillation. This was confirmed by extracting ground foliage with cold H_2O. An ethyl ether extract of this aqueous extract did not contain **1**, but hydro-distillation with concurrent extraction into hexane gave **1** in good yield.

The principal lactone precursor

In a search for a rapid assay to detect the presence of the precursor of lactone **1**, we found that heating a neutralized aqueous extract or fraction, then extracting with chloroform gave **1** plus a lesser amount of the

Table 1. NMR data for lactones*

	1	2	10		11		13		16		18
Position	(^{1}H)	(^{1}H)	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	(^{1}H)
2	—	—	—	176.9	—	172.3	—	176.9	—	175.1	—
3	6.13;*d*;5	6.20;*dd*;1,6	6.20;*dd*;2,6	123.0	6.18;*dd*;2,6	123.0	*ca* 2.6;*m*	24.1	6.22;*dd*;2,6	123.0	2.7–2.45;*m*
4	7.32;*d*;5	7.65;*br d*;6	7.46;*dd*;2,6	153.4	7.40;*dd*;2,6	152.6	*ca* 2.15;*m* *ca* 2.06;*m*	28.7	7.57;*dd*;1,6	153.1	2.4–2.32;*m*
5	—	—	4.94;*td*;2,5	87.1	5.06;*td*;2,4	83.9	4.35;*dt*;6,7	84.1	4.59;*ddd*;1,2,3	86.6	4.41;*dt*;3,7
6	5.33;*q*;7	5.80;*br dq*;1,7	3.93;*dq*;5,6	68.3	5.21;*dq*;4,7	68.1	3.79;*quint*;6	70.0	4.06;*dq*;3,6	67.8	4.13;*dq*;3,7
7	1.96;*d*;7	1.92;*d*;7	1.32;*d*;6	18.8	1.35;*d*;7	16.1	1.27;*d*;6	18.5	1.34;*d*;6	18.9	1.22;*d*;7
6	—	—	—	—	2.02	170.1,	—	—	—	—	—
-OAc						20.8					

*In $CDCl_3$; shifts in ppm; multiplicity (*s* = singlet, *d* = doublet, *q* = quartet, *br* = broad); couplings in Hz.

(*E*)-diastereomer (**2**) (^{1}H NMR data in Table 1). The amount of **1** could be estimated by GC. This assay was used to guide the isolation of the precursor from a methanol–chloroform foliage extract.

Partitioning this extract between H_2O and chloroform gave the precursor only in the aqueous phase. Treatment of the reddish-brown aqueous phase with charcoal removed much of the colour, but most of the precursor remained in solution. Evaporation yielded a reddish treacle, which was subjected to a variety of chromatographic fractionations, flash silica gel column chromatography proving to be the most convenient procedure for purifying the principal precursor of **1**.

This proved to be a substance, **A**, whose mass and ^{1}H and ^{13}C NMR spectra (Table 2) were consistent with a glycoside with the molecular formula $C_{12}H_{14}O_8$. The ^{1}H NMR signals of **A** were appropriate for a β-glucopyranoside, with ^{13}C NMR signals (correlated to the ^{1}H NMR signals by a HETCOR experiment) supporting this conclusion (Table 2) [9].

The ^{1}H and ^{13}C NMR spectra of **A** also showed signals typical of an α,β-unsaturated lactone, with further couplings to a CH(O).CH(O).CH_3 moiety (Table 2), i.e. a lactone glycoside of 4,5-dihydroxyhex-2-enoic acid. The possibility that **A** was the δ-lactone osmundalin (**5**), reported from ferns of the genus *Osmunda* [10, 11], was eliminated on the basis of the olefinic coupling constants. Therefore, it seemed that **A** was a glycoside of a 5-(1-hydroxyethyl)-2(5*H*)-furanone. We could find no reports of such glycosides. Only one of the four possible stereoisomeric 5-(1-hydroxyethyl)-2(5*H*)-furanones has been characterized, the 5*R*,6*S*-isomer (**6**), which has been reported as a hydrolysis product of **5** [10] and as a natural product [12]. ^{1}H NMR data have been reported for a mixture of the acetates (**7** and **8**) of (5*R*,6*S*)-and(5*S*,6*S*)-5-(1-hydroxyethyl)-2(5*H*)-furanones, synthesized from a mushroom metabolite [13]. The esters of all four 5-(1-hydroxyethyl)-dihydro-2-furanones ('soleroles') with (*R*)-(+)-Mosher's acid have been described: the ^{1}H NMR data for the diastereomeric esters were significantly different [14].

Treatment of **A** with a β-D-glucosidase released an aglycone (**10**) whose ^{1}H NMR data (Table 1) were similar to, but different from, those reported for **6** [10, 12]. In particular, our chemical shifts for both H-4 and

Table 2. NMR data for lactone glycosides*

	9(A)		12		15(B)		17	
Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	—	176.4	—	184.7	—	176.6	—	185.0
3	6.28;*dd*;2,6	121.5	2.66;*m*	26.4	6.24;*dd*;2,6	121.7	2.66;*m*	23.8
4	7.79;*dd*;1,6	156.7	2.36;*m* 2.2;*m*	31.3	7.76;*dd*;1,6	155.6	2.37–2.1;*m*	31.1
5	5.35;*ddd*;1,2,5	86.4	4.68;*m*	86.8	5.38;*ddd*;1,2,3	87.1	4.75;*m*	86.8
6	4.30;*dq*;5,6	74.2	4.10;*m*	75.7‡	4.34;*dq*;3,7	75.3	4.25;*dq*;3,6	75.9‡
7	1.29;*d*;6	15.0	1.27;*d*;6	17.4	1.18;*d*;7	14.0	1.23;*d*;6	16.9
1′	4.55;*d*;8	100.6	4.56;*d*;8	102.6	4.50;*d*;8	101.7	4.52;*d*;8	103.9
2′	3.25;*dd*;8,9	72.8	3.1–3.9;*m*†	72.4‡	3.19;*dd*;8,9	72.9	3.25;*dd*;8,9	72.2‡
3′	3.49;*t*;9	69.5	3.1–3.9;*m*†	78.5‡	3.44;*t*;9	69.4	3.7–3.3;*m*†	78.4‡
4′	3.38;*t*;9	75.7	3.1–3.9;*m*†	78.6‡	3.38;*m*†	75.6	3.7–3.3;*m*†	78.5‡
5′	3.41;*ddd*;2,6,9	75.9	3.1–3.9;*m*†	78.7‡	3.38;*m*†	75.9	3.7–3.3;*m*†	79.0‡
6′	3.91;*dd*;2,12 3.72;*dd*;6,12	60.6	3.1–3.9;*m*†	63.5	3.84;*dd*;2,12 3.67;*dd*;5,12	60.6	3.7–3.3;*m*†	63.2

*In D_2O; shifts in ppm; multiplicity (*s* = singlet, *d* = doublet, *q* = quartet, *br* = broad); couplings in Hz.

†Unresolved.

‡Assignments interchangeable within columns.

H-6 were more than 0.1 ppm upfield of those reported for **6**. This ruled out the (5*R*,6*S*) and (5*S*,6*R*) possibilities. As the circular dichroic spectrum of **A** was the near mirror image of that of **4** [15], which has the (5*S*)-configuration, it was proposed that **A** was **9**, the β-D-glucopyranoside of (5*R*,6*R*)-5-(1-hydroxyethyl)-2(5*H*)-furanone (**10**). This was supported by acetylation of aglycone **10** to give a product (**11**) whose ^{1}H NMR resonances (Table 1) matched with one set of the values reported for the mixture of the (5*R*,6*S*)- and (5*S*,6*S*)-acetates (**7** and **8**) [13], i.e. the (5*S*,6*S*)-isomer (**8**).

Verification of the structure of **A** was achieved by hydrogenation to a dihydro-derivative (**12**) (NMR data in Table 2) from which the aglycone (**13**) (NMR data in Table 1) was released by treatment with β-D-glucosidase. The aglycone was then esterified with (*R*)-(+)-Mosher's acid to give a preparation with ^{1}H NMR data matching the values reported for the ester (**14**) of (5*R*,6*R*)-5-(1-hydroxyethyl)-2(5*H*)-furanone [14]. Therefore, the dihydro-aglycone is **13** and the major lactone glycoside **A** is **9**.

Other lactone glycosides

Accompanying **9** in the later fractions from column chromatography was a second substance, **B**, which also proved to be a precursor of lactone **1**. Careful chromatography of these mixtures afforded pure **B**, whose ^{1}H and ^{13}C NMR spectra (Table 2) were consistent with a diastereomer of **9**. Treatment with β-D-glucosidase released an aglycone (**16**) whose ^{1}H and ^{13}C NMR data (Table 1) were very similar to those reported for **6** [10, 12]. The (5*S*,6*R*)-configuration for **B**, i.e. **15**, was inferred as the circular dichroic spectrum of **B** resembled that of **4** [15], which has the (5*S*)-configuration. Hydrogenation of **B** gave a dihydro-derivative (**17**) whose aglycone gave ^{1}H NMR data corresponding to those reported for the (5*R*,6*S*)-stereomer [10]. Its identity as the enantiomeric (5*S*,6*R*)-compound (**18**) was confirmed by making the previously described (*R*)-(+)-Mosher's ester (**19**) [14].

A concern that one or other of **9** or **15** might itself be an artefact produced by epimerization at C-5 was allayed when warming either **9** or **15** in D_2O and pyridine-d_5 resulted in no isomerization (^{1}H NMR). Remarkably, elimination to **1** and **2** occurred without any indication of H/D exchange. Both **9** and **15** gave the same mixture of **1** and **2**. Elimination of glucose from these glycosides was surprisingly facile: solutions in D_2O were stable at room temperature for days, while those containing the NMR reference sodium 3-trimethylsilylpropanoate-d_4 (TSP-d_4) slowly released **1**. This behaviour parallels the release of protoanemonin when ranunculin is heated with sodium acetate [8].

Dihydro-derivatives **12** and **17** appeared at the tail of the fractions containing the lactone precursors **9** and **15**. This recalls the co-occurrence of **6** and its dihydro-derivative in *Osmunda japonica* [12]. Both **12** and **17** were stable in H_2O in the presence of weak bases.

Phenolic glycosides

Apart from the lactone precursors and their dihydro-derivatives, the chromatographic separations of the *H. biformis* aqueous extract also afforded, as major constituents, sucrose and two known phenolic glycosides: picein (piceoside) (**20**) and pyroside (arbutin 6-*O*-acetate) (**21**) [16, 17]. A small amount of arbutin (**22**) was also isolated.

Occurrence of glycosides in Halocarpus *foliage*

This principal lactone precursor **9** was present in the bulk sample of *H. biformis* juvenile foliage at the remarkably high level of *ca* 50 mg g^{-1} dry foliage. This high level meant that the characteristic ^{1}H NMR signals of **9** could be detected in a D_2O solution prepared from a crude ethanol extract, which proved that **9** was not an artefact produced by the isolation procedures. We used this NMR method to check for the presence of **9**, and other glycosides, in different collections and foliage types of all three *Halocarpus* species (Table 3). Lactone glycoside **9** was present in juvenile *H. biformis* foliage from two different geographic locations, whereas **9** was not detected in adult *H. biformis* foliage. This supported our earlier observation of lactone **1** in hydro-distilled extracts from juvenile foliage of five different *H. biformis* plants, whereas adult foliage from the same plants yielded no detectable **1** [3]. Lactone glycoside **9** was present in one of two extracts of juvenile *H. bidwillii* foliage, whereas in previous work, **1** was not detected in hydro-distilled extracts of nine different plants from two geographic locations [7]. Glycoside **9** was also present in three out of four *H. kirkii* samples, including one of adult foliage. There does not seem to be any pattern to the occurrence of **9** in these *Halocarpus* foliage samples, but no attempt was made to control variables such as seasonal variation. Presumably, the H_2O-soluble **9** can be transported and metabolized by the plants.

The phenolic glycosides pyroside (**21**) and picein (**20**) were found at high levels in most of the *Halocarpus* foliage extracts (Table 3). These compounds have not been reported before from *Halocarpus* or from any other New Zealand gymnosperms [1, 2]. The presence of **21** may be related to the colour change of ground foliage to red-brown, since the structurally similar **22** is involved in blackening of *Pyrus* leaves [18]. A *p*-diphenol oxidase has been detected in all three *Halocarpus* species [19].

Biological activity of lactone glycosides

We tested lactone glycosides **9**, **12** and **15** for cytotoxic and antimicrobial activities, but did not detect any. This contrasts with the biological activities of other lactone glycosides in the same assays. The simpler homologue **4** was quite strongly cytotoxic (unpublished results) and a 2(5*H*)-furanone glycoside from a different New Zealand gymnosperm showed antimicrobial activity [20].

Table 3. Glycosides in *Halocarpus* foliage*

Species	Foliage type†	Collection code‡	Glycoside level§		
			Lactone (**9**)	Pyroside (**21**)	Picein (**20**)
H. biformis	J + A	920121-06	−	+ +	+
H. biformis	J	920121-07	+ +	+ +	+
H. biformis	A	920929-09	−	+ +	+
H. biformis	J	930608-03	+ +	+	+ +
H. biformis	J	930609-10	+	+	+ +
H. bidwilli	J	920929-05	−	+	−
H. bidwilli	A	920929-06	−	+	−
H. bidwilli	J	930405-18	+	+ +	+
H. bidwilli	A	930405-19	−	+ +	+
H. kirkii	J	921007-02	+	+	+ +
H. kirkii	A	921007-03	−	+	+ +
H. kirkii	J	921023-01	+ +	−	+ +
H. kirkii	A	921023-02	+	+	+

*Determined by ^{1}H NMR spectroscopy of D_2O-solubles from EtOH extracts.
†A = adult; J = juvenile.
‡Code identifies collection date, e.g. 920121-06 was collected on 21 January 1992.
§+ + = Major component; + = present; − = not detected.

EXPERIMENTAL

General. Solvents were removed under red. pres. on a rotary evaporator, with the water-bath <35°. ^{1}H and ^{13}C NMR spectra were recorded at either 400 or 200 MHz. For samples dissolved in $CDCl_3$ the residual $CHCl_3$ resonance (δ_H 7.27 ppm) was used as a reference, while for ^{13}C spectra the solvent resonance (δ_C 77.0) was similarly used. For samples dissolved in D_2O, TSP-d_4 was used as int. reference (trimethylsilyl resonance δ_H and $\delta_C = 0$), except in a few cases (noted below) when the DOH signal was used ($\delta_H = 4.7$).

Plant material. Juvenile *H. biformis* foliage for the bulk extraction was collected from sub-alpine scrub on Mt. Cargill, altitude 600 m, grid reference 200790 (S164), in mid-January 1992. A voucher specimen, code 920121-07, has been kept in the Plant Extracts Research Unit. The sample was air-dried at room temp. in the laboratory for 1 week, then ground in a coffee mill. Other *Halocarpus* collections were made from the Dunedin Botanical Gardens (*H. biformis* and *H. bidwillii*); Kelly's Road, W. Auckland (*H. kirkii*); Otari Botanic Garden, Wellington (*H. kirkii*); Maungatua Scientific Reserve, near Dunedin (*H. bidwillii*); and Jackson River Rd South of Haast (*H. biformis*). Voucher specimens, with codes listed in Table 3, have been kept in the Plant Extracts Research Unit.

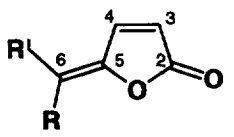
Assay procedures for lactone precursors. An aq. extract or fr. (1 ml) was neutralized to pH 7–8 with satd $NaHCO_3$ (aq.), heated to 100° for 30 min in a closed vial, then extracted with $CHCl_3$ (0.5 ml, containing 0.05% nonane). Extracts were analysed by GC on a DB-1 capillary column (9.5 m) with H_2 carrier gas (linear gas velocity 50 cm s^{-1}). Injections (0.5 μl) were split (100:1) at 260°, the temp.-programme was 50°–200° at 45° min^{-1}; and the FID was at 310°. Under these conditions, nonane had a R_t of 0.45 min, lactone **1** 1.22 min and lactone **2** 1.35 min.

An alternative, NMR-based assay procedure was also developed. To a sample of the *H. biformis* aq. extract (*ca* 50 mg), or a chromatographically purified fr. (*ca* 5 mg) in D_2O (1 ml) was added pyridine-d_5 (20 μl). This mixt. was heated for 10 min in a boiling-water bath, cooled, then examined by ^{1}H NMR. The presence of **1** was revealed by peaks at δ_H (DOH ref.) 1.80 (3H, *d*, $J = 7.5$ Hz) and 5.3 (1H, *q*, $J = 7.5$ Hz). The soln was extracted with $CDCl_3$ (1 ml), with centrifugation to separate the phases, and the ^{1}H NMR spectrum of the $CDCl_3$ extract was recorded. The resonances for **1** and **2** (*ca* 15% of **1**) are given in Table 1. It was possible to quantitate this assay by using neopentyl alcohol as int. standard in the $CDCl_3$ (typically at a concn of 2 mg ml^{-1}).

Bulk extraction and fractionation. Dried, ground *H. biformis* juvenile foliage (75 g) was stirred mechanically with MeOH–$CHCl_3$ (3:1, 667 ml) for 6 hr, then filtered. The filter-cake was washed with $CHCl_3$–MeOH (3:1, 100 ml), then resuspended in MeOH–$CHCl_3$ (3:1, 667 ml), stirred mechanically for 4 hr and allowed to stand at room temp. overnight. After filtration, and washing the filter cake with MeOH, the residual plant material was an amber powder (43.5 g after air-drying).

The combined extracts, after removal of solvents, gave a dark green gum (41 g). This was partitioned between $CHCl_3$ (100 ml) and H_2O (100 ml), and the $CHCl_3$ phase re-extracted with H_2O (2 × 50 ml). The combined aq. extracts were evapd to give a dark red treacle. The $CHCl_3$ extract was similarly evapd to give a dark green wax. GC assays for the lactone precursor revealed that this was present in the aq. extract, and not in the $CHCl_3$-soluble material.

The aq. extract was dissolved in H_2O (75 ml), animal charcoal (BDH technical, 15 g) was added, and the mixt. was first degassed under aspirator vacuum, then



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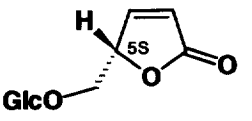
2 R = H, R' = CH_3
3 R = R' = H



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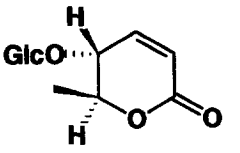

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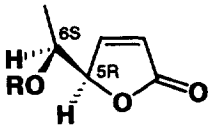

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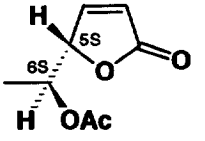
7 R = Ac



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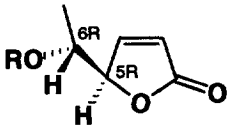

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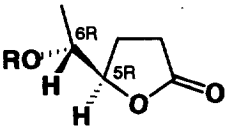
11 R = Ac



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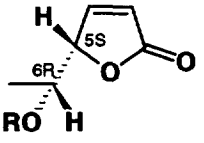
13 R = H
14 R = Mosher's acid



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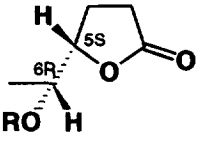
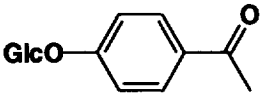
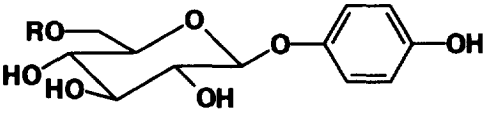
16 R = H



17 R = Glc
18 R = H
19 R = Mosher's acid



20



21 R = Ac
22 R = H

stirred at room temp. for 10 min before being filtered through a pad of Celite. The deep red filtrate was concd to a syrup. This was taken up in H_2O (75 ml) and the soln was treated with charcoal (15 g) as before. The filter cake was washed with H_2O (75 ml) and the combined filtrates and washes were evapd to give a red-brown treacle (9.8 g). Extraction of the combined charcoal and Celite filter cakes with MeOH (250 ml),

MeOH–H_2O (4:1, 100 ml) and again with MeOH (100 ml), followed by evapn of these extracts, gave an orange glass (4.2 g). GC assays for the lactone precursor revealed it to be concd in the aq. extract, with much less in the charcoal-adsorbed material.

A portion of this eq. *H. biformis* extract (*ca* 6 g) was triturated with H_2O (10 ml) and centrifuged. The dark red supernatant was removed with a Pasteur pipette, and the solids (largely pyroside) resuspended in H_2O (2 ml) and recentrifuged. The supernatant was withdrawn as before, combined with the first, and MeOH (30 ml) added. To this soln was added silica gel (230–500 mesh) and the solvents were removed to given an orange powder. This was loaded on to a column of the same grade of silica gel packed in EtOAc–MeOH–H_2O (200:27:20) and the column was then developed using the same solvent, collecting 53 × 25 ml frs. Finally, the column was stripped with MeOH–H_2O (1:1) (200 ml) and H_2O (500 ml). The individual frs were examined by TLC (*n*-BuOH–EtOH–H_2O, 4:1:1, with anisaldehyde–HOAc–H_2SO_4 detecting dip) and ^1H NMR (D_2O, ref. DOH).

Pyroside (**21**). Crystallized from frs 6–8, mp 216° (lit. mp 213° [17]); δ_H (D_2O) 7.02 (2H, *d*, $J = 9$ Hz), 6.86 (2H, *d*, $J = 9$ Hz) 4.96 (1H, *d*, $J = 8$ Hz, H-1′), 4.40 (1H, *dd*, $J = 2$, 12 Hz, H-6′), 4.36 (1H, *dd*, $J = 6$, 12 Hz, H-6′), 3.75 (1H, *ddd*, $J = 2$, 6, 10 Hz, H-5′), 3.5–3.65 (3H, *m*, H-2′, 3′, 4′), 2.10 (3H, *s*, Ac) [17]; δ_C(D_2O) 176.7 (*s*), 154.0 (*s*), 153.05 (*s*), 121.2 (2C, *d*), 118.9 (2C, *d*), 103.9 (*d*), 78.1 (*d*), 76.1 (*d*), 75.6 (*d*), 72.2 (*d*), 65.8 (*t*), 22.9 (*q*).

Picein (**20**). Obtained from frs 14–16 (590 mg) and recrystallized from EtOH, mp 185–190° (lit. mp 160–165° [17]); δ_H (D_2O) 8.00 (2H, *d*, $J = 9$ Hz), 7.20 (2H, *d*, $J = 9$ Hz), 5.25 (1H, *d*, $J = 7$ Hz, H-1′), 3.98 (1H, *dd*, $J = 2$, 12 Hz, H-6A′), 3.78 (1H, *dd*, $J = 6$, 12 Hz, H-6′B), 3.74–3.46 (4H, *m*, H-2′,3′,4′,5′), 2.62 (3H, *s*) [17]; δ_C (D_2O) 205.4, 163.7, 134.0 (2C), 121.3, 118.9 (2C), 102.2, 79.1, 78.3, 75.7, 72.2, 63.3, 28.9 (see ref. [16] for ^1H and ^{13}C NMR data in DMSO-d_6).

Picein was accompanied by small amounts of another glycoside. This was isolated by prep. TLC (silica gel 60 F_{254}, *n*-BuOH–EtOH–H_2O (4:1:1)) and shown by ^1H and ^{13}C NMR to be arbutin (**22**): δ_H (D_2O) 7.06 (2H, *d*, $J = 9$ Hz), 6.88 (2H, *d*, $J = 9$ Hz), 4.97 (1H, *d*, $J = 7$ Hz, H-1′), 3.93 (1H, *dd*, $J = 2$, 12 Hz, H-6′A), 3.77 (1H, *dd*, $J = 5$, 12 Hz, H-6′B), 3.4–3.65 (4H, *m*, H-2′,3′,4′,5′); δ_C (D_2O) 154.1, 153.2, 121.3 (2C), 119.1 (2C), 104.2, 78.9, 78.4, 75.8, 72.3, 63.4 (see ref. [16] for ^1H and ^{13}C NMR data in DMSO-d_6); identical with an authentic sample.

The principal lactone-precursor (**9**) appeared as the major component of frs 27–33, with increasing amounts of its diastereomer (**15**) in frs 34–40. Frs 41–53 contained increasing amounts of lactones **12** and **17**, followed by sucrose.

Principal lactone precursor, (5R,6R)-5-(1-*hydroxyethyl*)-2(5H)-*furanone* β-D-*glucopyranoside* (**9**). Frs from flash CC, which appeared to be nearly pure, as judged by ^1H NMR, were further purified by percolation through a plug of PVP, to remove an orange pigment. The aq. extract was then stirred with animal charcoal (*ca* 1/5th the wt of original fr. wt) for 3 min and filtered through Celite. The solids were washed with cold H_2O and the filtrate and washings were then evapd to give **9** as a glass: FABMS *m/z* 291 $[M + H]^+$, 313 $[M + Na]^+$; UV (H_2O) λ_{max} 275 nm (ε 800); IR (KBr disc) 3400, 1750, 1654 cm^{-1}; NMR data: Table 2. The CD spectrum in H_2O was antipodal to that of ranunculin [15].

(5R,6R)-5-(1-*Hydroxyethyl*)-2(5H)-*furanone* (**10**). Glycoside **9** (104 mg) was dissolved in 0.1 M acetate buffer (pH 5, 7 ml), β-glucosidase was added, and the mixt. was stirred at room temp. After 4 hr an indicator strip (blood sugar test) revealed that Glc had been released. $CHCl_3$ extracts (5 × 5 ml) were dried (Na_2SO_4) and evapd under a N_2 jet to give **10** as an oil (12 mg): NMR data: Table 1. The CD of this material in MeOH was antipodal to that of ranunculin [15].

(5R,6R)-5-(1-*Hydroxyethyl*)-2(5H)-*furanone acetate* (**11**). The acetate of **7** was prepd in the usual way with Ac_2O and pyridine to give **11** as an oil; NMR data: Table 1.

The β-D-*glucopyranoside of* (5R,6R)-5-(1-*hydroxyethyl*)-*dihydro*-2-*furanone* (**12**). To a soln of **9** (70 mg) in 95% EtOH was added 5% Pd on $CaCO_3$ (100 mg), and the mixt. was stirred under an atmosphere of H_2 for 3 hr. The catalyst was removed by filtration through Celite and the solids washed with MeOH (3 ml). The combined filtrate and washings were then evapd to a residual gum. This was dissolved in D_2O (1 ml) and solvent again removed under red. pres. to given **12** as a glass; NMR data: Table 2. Frs 41–56 from the flash CC of *H. biformis* aq. extracts had identical spectrometric properties to this synthetic sample of **12**.

(5R,6R)-5-(1-*Hydroxyethyl*)-*dihydro*-2-*furanone* (**13**). Glycoside **12** (133 mg) was treated with β-glucosidase to give **13** as an oil (8 mg); NMR data: Table 1.

The ester (**14**) *of* **13** *with* R-(+)-*Mosher's acid*. To *R*-(+)-Mosher's acid (26 mg) in dry hexane (3 ml) containing dry DMF (8.4 mg) was added oxalyl chloride (70 μl) (procedure from ref. [21]). A rapid reaction ensued and a white solid sepd. After 1 hr, the reaction mixt. was centrifuged, the supernatant removed with a Pasteur pipette, and evapd to a residual oil. This was dissolved in $CDCl_3$ (0.5 ml) and added to **13** (6.2 mg) in $CDCl_3$ (1 ml) containing dry Et_3N (7.5 μl) and DMAP (*ca* 2 mg). A reaction took place immediately. After 1 h the mixt. was washed with 1 M aq. H_2SO_4 (3 ml) and then with satd aq. $KHCO_3$ (3 ml). The $CDCl_3$ was sepd, dried ($MgSO_4$) and evapd to small vol. (*ca* 0.5 ml). The ^1H NMR data matched the reported values for the (5*R*,6*R*)-ester [14].

The minor lactone precursor, (5S,6R)-5-(1-*hydroxyethyl*)-2(5H)-*furanone* β-D-*glucopyranoside* (**15**). Frs 41–43 (150 mg) from flash CC of the *H. biformis* aq. extract were combined in H_2O (2 ml) and the soln percolated through PVP to remove orange pigment. After washing the solids with H_2O (5 ml) the combined filtrate and washings were evapd to a glass. This was

taken up in MeOH and TLC-grade silica gel 60 (0.5 g) was added. The solvent was removed and the powder obtained was then packed into a column of the same grade of silica gel. The column was developed with EtOAc–MeOH–H_2O (200:27:20) in 5-ml portions. Compound **9** appeared in frs 8 and 9, while frs 12 (12 mg) and 13 (9 mg) were nearly homogeneous prepns of **15** (as revealed by ^1H NMR), which was obtained upon evapn as a glass; NMR data: Table 2. The CD of this material, in H_2O, was nearly superimposable upon that of ranunculin [15].

(5S,6R)-5-(1-*Hydroxyethyl*)-2(5H)-*furanone* (**16**). Treatment of fr. 14 from the above silica column (18 mg) with β-glucosidase gave **16** as an oil (3 mg); NMR data: Table 1.

The β-D-*glucopyranoside of* (5S,6R)-5-(1-*hydroxyethyl*)-*dihydro*-2-*furanone* (**17**). Fr. 12 from the above silica column (12 mg) was dissolved in H_2O (3 ml), to which 5% Pd on $CaCO_3$ (2 mg) was then added, and the mixt. was stirred under H_2 for 3 hr. The catalyst was removed by filtration through Celite, and the filtrate and washings were evapd under red. pres. to give **17** as a glass (12 mg); NMR data: Table 2.

(5S,6R)-5-(1-*Hydroxyethyl*)-*dihydro*-2-*furanone* (**18**). Glycoside **17** (12 mg) was treated with β-glucosidase as before to give **18** as an oil (4 mg); NMR data: Table 1.

The ester (**19**) *of* **18** *with* R-(+)-*Mosher's acid*. Ester **19** was prepd from **18** as before, except that Et_3N was replaced by pyridine [21]. The ^1H NMR spectrum matched that reported for the (5*S*,6*R*)-derivative [14].

Survey of glycosides in Halocarpus *collections*. Foliage samples (leaves and terminal twigs) were air-dried and ground. Sub-samples (5 g) were extracted by shaking with EtOH [rectified spirits, ethanol–H_2O (19:1); 50 ml] for 24 h. After filtration, extracts were stored at −15°. Sub-samples (1 ml) were dried (Speed-vac), dissolved in H_2O and dried again, then dissolved in D_2O (1 ml), and filtered into NMR tubes. ^1H NMR spectra, with solvent suppression, were examined for the signals of the glycosides reported above (results in Table 3).

Biological assays. Details of antimicrobial and cytotoxicity assays published elsewhere [22]. Glycosides **9**, **12** and **15** were inactive against *Bacillus subtilis*, *Candida albicans* and *Trichophyton mentagrophytes* at 150 μg $disc^{-1}$; they were not cytotoxic to BSC-1 cells at 150 μg $disc^{-1}$, and had IC_{50} >62.5 mg ml^{-1} against P388 leukaemia cells.

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