Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 1953–1964

BIOORGANIC & MEDICINAL CHEMISTRY

The Synthesis and Antibacterial Activity of Totarol Derivatives. Part 1: Modifications of Ring-C and Pro-Drugs

Gary B. Evans,[a,*] Richard H. Furneaux,[a] Michael B. Gravestock,[b] Gregory P. Lynch[a] and G. Kenneth Scott[c]

[a]*Industrial Research Limited, PO Box 31-310, Lower Hutt, New Zealand*
[b]*ZENECA Pharmaceuticals PLC, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*
[c]*School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

Received 11 February 1999; accepted 5 April 1999

Abstract—A series of analogues of, and potential pro-drugs derived from, the potent antibacterial diterpene totarol (**1**) were synthesized in order to elucidate the minimum structural requirements for antibacterial activity and to seek compounds with good bioavailability in vivo. These analogues varied in the structural features of their aromatic rings and the prodrugs were *O*-glycosylated derivatives. They were tested in vitro against three Gram-positive bacteria: β-lactamase-positive and high level gentamycin-resistant *Enterococcus faecalis*, penicillin-resistant *Streptococcus pneumoniae*, and methicillin-resistant *Staphylococcus aureus* (MRSA); and against the Gram-negative multi-drug-resistant *Klebsiella pneumoniae*. None of the analogues was more potent than totarol itself, which is effective against these Gram-positive bacteria at MIC values of 7 μM. The results were evaluated in terms of a structure–activity relationship and this showed that a phenolic moiety was essential for potent antibacterial activity. Amongst the pro-drugs, totaryl α-D-mannopyranoside (**22**) proved the most active in vitro (MIC 18 μM). The in vivo antibacterial activities of compounds **1**, **22** and totarol β-lactoside **(23)** were assessed in a mouse model of infection, but they were found to be ineffective. Compounds **1** and **22** were shown to be cytotoxic towards proliferating human cell cultures, CH 2983, HeLa, and MG 63, but only at concentrations of >30 μM.

Introduction

Since the early 1950s and the emergence of penicillin-resistant Staphylococci, the incidence of infections caused by bacteria resistant to commonly used antibiotics has increased alarmingly.[1,2] In particular, resistances to multiple antibiotics of strains of Gram-positive bacteria such as *Enterococcus faecalis*, methicillin resistant *Staphylococcus aureus* (MRSA),[3] and *Streptococcus pneumoniae*[4] are significant problems amongst hospitalized patients. Totarol (**1**),[5] a naturally occurring phenolic diterpene, is the major chemical constituent in the hexane extract of the heartwood of the New Zealand native tree *Podocarpus totara* G. Benn,[6,7] and can also be isolated from a variety of other sources.[5] It has been shown to have antibacterial activity against Gram-positive bacteria[8–14] and, in particular, MRSA (with an MIC of 2.7 μM in vitro).[3]

We now report the syntheses of the systematically modified analogues of totarol **2**–**23**. Their antibacterial activity was evaluated in vitro in an attempt to define the influence of structural features on the aromatic ring upon antibacterial potency in this class of diterpenes. In addition, the in vivo antibacterial activities of totarol (**1**) and two glycosylated analogues **22** and **23** were evaluated in a mouse infection model, and totarol (**1**) and totaryl α-D-mannopyranoside (**22**) were screened against a variety of human cell cultures in vitro in an effort to gauge the cytotoxicity of this class of antibiotics.

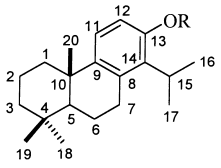
Results and Discussion

Synthesis of homologues 2–5 and substitution analogues 6–8

Totarol (**1**) has some structural features in common with previously reported diterpene antibiotics such as ferruginol (**10**), but inhibits the growth of Gram-positive bacteria at lower concentrations. One striking feature of totarol is that its phenolic and isopropyl groups

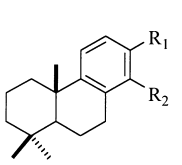
Key words: Antibacterials; glycosides; natural products; terpenes and terpenoids.
* Corresponding author. Tel.: +64-45690040; fax: +64-45690055; e-mail: g.evans@irl.cri.nz

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00162-5

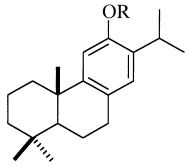


	R
1	H
11	Me
12	Ac
13	Tf
14	β-D-Glc*p*-$(OAc)_4$
15	α-D-Gal*p*-$(OAc)_4$
16	β-D-Gal*p*-$(OAc)_4$

	R
17	α-D-Man*p*-$(OAc)_4$
18	β-D-Lac*p*-$(OAc)_7$
19	β-D-Glc*p*
20	α-D-Gal*p*
21	β-D-Gal*p*
22	α-D-Man*p*
23	β-D-Lac*p*



	R_1	R_2
2	OH	H
3	OH	Me
4	OH	Et
5	OH	Pr
6	OH	OH
7	H	*i*-Pr
8	H	H



	R
9	Bz
10	H

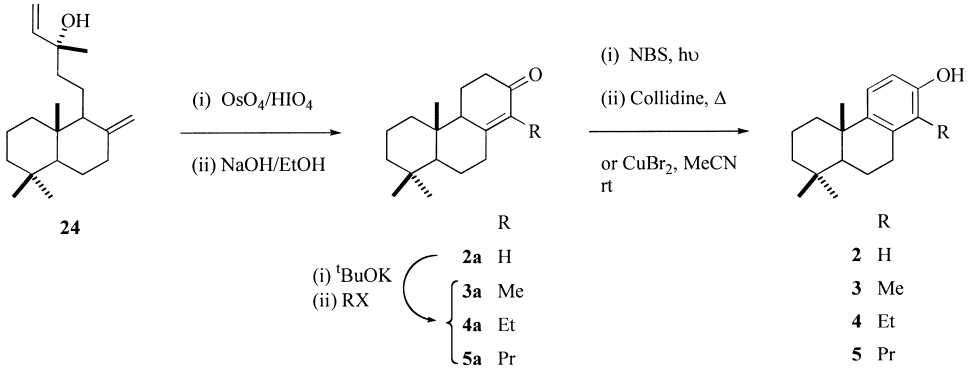
are in a sterically crowded environment.[5] The isopropyl group at C-14 'scissors' the phenolic group at C-13 in consequence of a non-bonding interaction between the former and the methylene group at C-7.[15] Manifest evidence of this steric shielding is seen in the IR spectrum of liquid **1** in which the OH stretching absorption is at 3500 cm^{-1} rather than 3300 cm^{-1} as observed for phenols, suggesting that intermolecular hydrogen bonding is not possible.[16]

Also the signals for C-16 and C-17 in the ^{13}C NMR spectrum of **1** are shifted upfield relative to the corresponding signals for similar diterpenes such as ferruginol, due to the greater proximity of these atoms to the phenolic group in the former case.[17] The first challenge was to assess whether this steric shielding might be the critical factor in totarol's greater antibacterial potency.

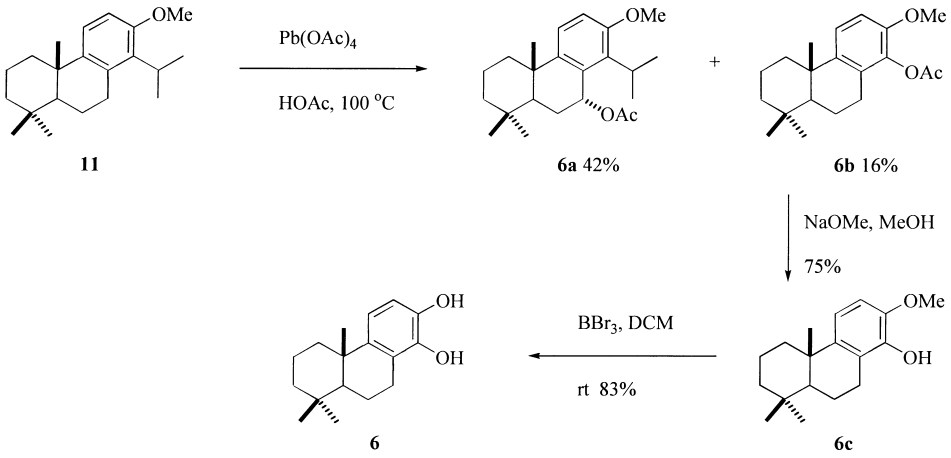
Firstly, the totarol analogue **2**, in which the isopropyl group at C-14 is replaced by a hydrogen atom, was synthesized. Manool (**24**) can easily be converted in two steps into enone **2a**[18] (Scheme 1),[19,20] treatment of which with *N*-bromosuccinimide in carbon tetrachloride under a heat lamp afforded a crude brominated product. Reaction in refluxing collidine gave the desired analogue **2**[21] in 65% overall yield.

Totarol analogues varying in the type of alkyl substituent at C-14 were then prepared. When enone **2a**[19] was heated under reflux in *tert*-butanol with potassium *tert*-butoxide followed by the separate dropwise addition of various alkyl halides,[22,23] the C-14 *n*-alkyl derivatives **3a**–**5a** were obtained (Scheme 1). Attempted aromatization of these substituted enones under the conditions described above for the conversion of **2a** into **2** was unsuccessful. Instead, a newer method which uses copper(II) bromide in acetonitrile at room temperature[24] was used to give the desired phenolic compounds **3**–**5** in 71, 64 and 80% yields, respectively.

The C-14 isopropyl group of totarol was then replaced by an hydroxy group. Treatment of *O*-methyl-totarol (**11**) with lead tetraacetate in acetic acid at 100°C afforded two chromatographically separable products, 7α-acetoxy-13-methoxytotara-8,11,13-triene (**6a**) and 14-acetoxy-13-methoxypodocarpa-8,11,13-triene (**6b**) in 42 and 16% yield, respectively (Scheme 2). The replacement of the C-14 isopropyl group by an acetoxy group in **6b** was confirmed by ^1H and ^{13}C NMR spectroscopy [AcO group present, δ_H 2.31; δ_C 168.9 (s), 20.5 (q); H-11, H-12 intact; *i*-Pr absent]. Saponification of **6b** using sodium methoxide in methanol followed by acidification gave compound **6c** in 75% yield, and demethylation of



Scheme 1.



Scheme 2.

this product using BBr_3[25] in CH_2Cl_2 afforded the catechol **6** in 83% yield.

Deoxygenation of totarol (1) was effected by reduction of its triflic ester **13**. Hydrogenolysis was achieved under one atmosphere of hydrogen over Pd/C in CH_2Cl_2 with a stoichiometric amount of triethylamine to prevent poisoning of the catalyst,[26] the reaction proceeding smoothly at room temperature and affording the known compound **7**[27] in 96% yield.

Compound **8**, in which the C-13 phenolic and the C-14 isopropyl groups of totarol are each replaced by a hydrogen atom, was prepared from podocarpic acid (**25**) which was converted to podocarpa-8,11,13-trien-12-ol (**8a**) by a literature procedure.[28] The latter was esterified with trifluoromethanesulfonic anhydride to afford the aryl triflate **8b** (65% yield) hydrogenolysis of which gave the desired podocarpa-8,11,13-triene (**8**)[29] in 91% yield (Scheme 3).

Synthesis of the positional isomer of totarol, ferruginol (10)

Ferruginol (**10**) is the major constituent of *Podocarpus ferrugineus* D. Don,[30] and its antimicrobial[31] and antibacterial[13,32–35] activities have been reported. It was selected for evaluation because the steric hindrance about the phenolic group differs significantly from that in totarol (**1**). Ferruginol (**10**) was obtained in 88% yield from ferruginyl benzoate, prepared by a literature procedure,[30] by saponification using sodium methoxide in methanol followed by acidification.

Alkylation and esterification of totarol (1)

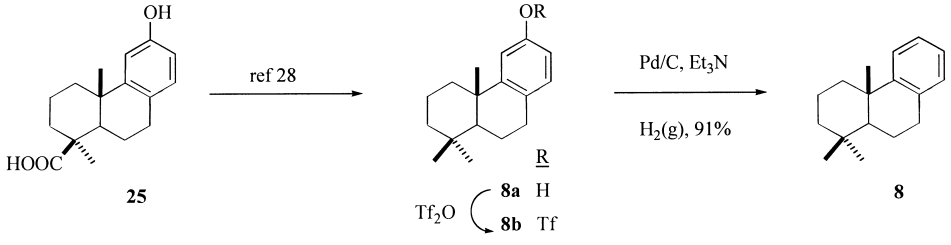
To gauge the contribution of the phenolic proton to the antibacterial activity of totarol (**1**), the phenol was *O*-substituted by methylation and acetylation to give *O*-methyl totarol (**11**) and totaryl acetate (**12**), respectively, by standard methods.[36] The triflate ester **13** of totarol (**1**) is referred to above.

Glycosylation of totarol (1)

As well as providing a means of further gauging the importance of the phenolic proton to totarol's antibacterial activity, the glycosylation of totarol (**1**) also provided a route to pro-drug derivatives with improved solubility in water and potentially better bioavailability. Glycosylation was achieved by the reaction of **1** with peracetylated glycosyl bromides[37] in the presence of silver triflate[38,39] which gave the peracetylated β-D-glucopyranoside (**14**), α-D-mannopyranoside (**17**) and β-lactoside (**18**) and a separable mixture of the peracetylated α- and β-D-galactopyranosides, **15** and **16**, in good to moderate yields. The anomeric configurations of the products were assigned on the bases of their ^1H NMR $^3J_{1,2}$ values.[40] Deacetylation using sodium methoxide in methanol yielded the five glycosylated totarols **19**–**23**.

Evaluation of in vitro antibacterial activities and their relationship to structural features on the aromatic ring

The antibacterial activities of totarol (**1**) and the compounds **2**–**23** were evaluated at concentrations of 2, 8



Scheme 3.

and 32 μg mL^{-1} against three Gram-positive bacteria: β-lactamase positive and high level gentamycin-resistant *E. faecalis*, penicillin-resistant *S. pneumoniae*, and methicillin-resistant *S. aureus* (MRSA); and the Gram-negative bacterium, multi-drug-resistant *K. pneumoniae*. The observed minimum inhibitory concentrations are listed in Table 1.

None of the compounds tested was active against *K. pneumonia*. Gram-negative bacteria have significant lipid components in their cell wall, up to 25% of dry weight, whereas the lipid content of Gram-positive bacterial cell walls is much smaller, 0–2.5%. It is thought that the former are protected from lipophilic antibacterial compounds, such as totarol, simply because these are trapped in the cell wall lipids, and do not reach the cell membrane.[41]

Totarol (**1**) and several of its analogues, notably **2**–**6** and **10**, were active against all three of the Gram-positive bacteria. None of the compounds displayed greater potency than totarol, though some were equipotent. Replacement of the C-14 isopropyl group of totarol with a linear alkyl group (Me, Et, or Pr) should somewhat reduce steric crowding of the adjacent phenolic group, but the antibacterial potency against Gram-positive bacteria was not reduced, homologues **3**–**5** exhibiting MIC's comparable with that of **1**. Replacement of the C-14 isopropyl group with a hydrogen atom or a hydroxy group, however, as in compounds **2** and **6**, respectively, resulted in a significant loss in activity (4- to 16-fold). This could be interpreted as being a result of either the phenolic group not being as sterically shielded as when an alkyl group is present at C-14, or of the reduction in the overall lipophilic character of these compounds.

Table 1. In vitro antibacterial activity MIC[a], μg mL^{-1} (values in brackets are μM)

Compound	*Enterococcus faecalis*	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
1	2 (7)	2 (7)	2 (7)	> 32
2	8 (33)	8 (33)	8 (33)	> 32
3	2 (8)	2 (8)	2 (8)	> 32
4	2 (7)	2 (7)	2 (7)	> 32
5	2 (7)	2 (7)	2 (7)	> 32
6	32 (120)	8 (31)	32 (120)	> 32
7	> 32	32 (120)	> 32	> 32
8	> 32	> 32	> 32	> 32
9	> 32	> 32	> 32	> 32
10	8 (28)	2 (7)	8 (28)	> 32
11	> 32	8 (27)	> 32	> 32
12	> 32	8 (24)	> 32	> 32
13	> 32	> 32	> 32	> 32
14	> 32	> 32	> 32	> 32
16	> 32	> 32	> 32	> 32
17	> 32	> 32	> 32	> 32
19	8 (18)	32 (71)	32 (71)	> 32
20	32 (71)	8 (18)	32 (71)	> 32
21	> 32	8 (18)	> 32	> 32
22	8 (18)	8 (18)	8 (18)	> 32
23	> 32	32 (51)	> 32	> 32

[a] Minimum inhibitory concentrations.

Ferruginol (**10**) is an isomer of totarol (**1**) in which the isopropyl and phenolic groups are transposed by one carbon atom around the aromatic ring. The C-7 methylene group in **10** no longer causes the isopropyl group to scissor the phenolic group, which is consequently not as sterically shielded as in totarol (**1**).[15] Ferruginol's antibacterial activity is reduced by up to a factor of 4 relative to that of totarol for two of the Gram-positive bacteria, but not the third.

Substitution of totarol's C-13 phenolic group with a hydrogen atom has a more dramatic effect, compound **7** inhibiting only *S. pneumoniae*, and at an MIC of 32 μg mL^{-1}. The residual activity of **7** against *S. pneumoniae* in the absence of a phenolic group, is notable however. Replacement of both the C-13 phenolic and C-14 isopropyl groups with hydrogen atoms gives compound **8** which exhibits no in vitro activity against any of the Gram-positive strains even at 32 μg mL^{-1}.

Methyl ether **11**, acetate **12** and triflate **13** were inactive, with the exceptions that **11** and **12** have MICs of 8 μg mL^{-1} against *S. pneumoniae*. Perhaps this organism can cleave the substituent groups of these derivatives to release totarol. It is noteworthy that this bacterium is most sensitive to several of the totaryl glycosides that were tested (**19**–**23**).

Overall, the presence of a phenolic group appears to be essential for potent activity against Gram-positive bacteria in this class of diterpenes, and it is noticeable that the most active compounds examined (**1**, **3**–**5** and **10**) all possess a primary or secondary alkyl substituent in the *ortho*-relationship to the hydroxy function. Since such *o*-alkylphenols oxidise to *o*-quinone methides[42] it may be concluded that the bioactive compounds studied here could manifest their activities by acting as antioxidants, which conclusion has previously been drawn for totarol itself on the basis of the findings of several biochemical experiments.[43] [The authors gratefully acknowledge the advice of a referee on this last point.]

Glycosylation of totarol (**1**) gave the potential pro-drugs **14**–**23** some of which exhibited in vitro antibacterial activity comparable with that of totarol. The α-D-mannopyranoside (**22**) in particular showed good activity against the three Gram-positive organisms, and the α-stereochemistry at the anomeric centre of the sugar moiety may be significant since the α-D-galactopyranoside **20** is more active than the corresponding β-anomer **21**. None of the peracetylated derivatives (**14**–**17**) showed any antibacterial activity in vitro. We speculate that the active species is totarol itself, released by endogenous bacterial glycosidases which are inhibited by *O*-acetylation of the substrates.

In vivo antibacterial activity of compounds 1, 22 and 23 in a mouse infection model

The in vivo activity of compound **1** was assessed in a mouse model of infection arising from a potentially lethal intraperitoneal challenge of methicillin susceptible *S. aureus* (MSSA). In an initial test, a subcutaneous

dose of totarol (**1**) was found to be protective, at a PD50 value of 7.1 mg/kg (Table 2). However, this result was not reproduced in two subsequent experiments, presumably because totarol is poorly bioavailable. We reasoned that totarol α-D-mannopyranoside (**22**) and totarol β-lactoside (**23**) might show improved in vivo activity due to greater bioavailability. Partition coefficient values between water and octanol were calculated for totarol, the totaryl glycoside (**22**) and the totaryl disaccharide glycoside (**23**) giving log *P* values of 7.5, 4.9 and 2.6, respectively. Clearly totarol in its unionized form is very lipophilic, and this can be somewhat ameliorated by glycosylation. Unfortunately, neither of the two glycosides **22** and **23** exhibited improved protective activity in the mouse model of infection (Table 2).

Table 2. In vivo antibacterial activity

Compound	Route[a]	PD_{50} mg kg^{-1}
Totarol (**1**)	sc	7.1
Totarol (**1**)	sc	> 16
Totarol (**1**)	sc	> 32
Glycoside (**22**)	sc	40
Glycoside (**23**)	sc	> 40
Vancomycin	sc	0.7

[a] sc = Subcutaneous injection.

In vitro cytotoxicity of compounds 1 and 22

To further assess the potential for this class of antibiotic for use in human medicine, the cytotoxicity of representative compounds was assessed against three human cell lines in culture. Initially, totarol (**1**) and totaryl α-D-mannopyranoside (**22**) were tested at concentrations of 25, 5, 1 and 0.2 μg mL^{-1}. It was soon clear that both test compounds were exerting adverse effects on cell growth at the highest concentrations. Within 24 h of addition of the compounds, the cells became rounded and detached from the plates. This was true for all three cell types, and was confirmed when test cultures were fixed, stained and measured. Test compound concentrations of 25 μg mL^{-1} resulted in cell growth values ranging from 4% (±1%) to 25% (±3%) for the three cell types, relative to control culture values set at an arbitrary 100% (±11%).

We deduced that both totarol (**1**) and compound **22** were exerting cytotoxic or growth-inhibitory effects on human cells at concentrations between 5 and 25 μg mL^{-1}. A second series of experiments were performed to define the effective concentrations more closely. The test solutions had concentrations of 21, 14 and 7 μg/mL and the results are summarized in Table 3.

For totarol, 50% growth inhibition occurred at concentrations between 14 and 21 μg mL^{-1} (49–73 μM) for each of the three cell lines tested. In the case of compound **22**, the corresponding point in the concentration range tested was between 21 and 25 μg mL^{-1} (see previous experiment; 47–56 μM), except in the case of the human fibroblast line (CH 2983), where 50% growth inhibition apparently occurred between 14 and 21 μg mL^{-1} (31–47 μM).

Table 3. Cytotoxicity of compounds **1** and **22**

Compd	[μg mL^{-1}]	[μM]	Cell growth (%)		
			CH 2983	HeLa	MG 63
1	21	73	36	13	15
1	14	49	103	96	97
1	7	25	115	106	105
22	21	47	33	85	102
22	14	31	118	98	110
22	7	16	100	116	95

An apparent difference in the sensitivity of the two compounds largely disappears when molar concentrations are compared. It can be concluded that the human cells tested grow normally at concentrations of 30 μM or less of either totarol or its glycoside **22**.

The nature of the growth-inhibitory effect has not been explored, except to examine the possible cytotoxic effect of totarol (**1**) on confluent, quiescent cells. Compounds **1** and **22** were tested at concentrations of 21, 14 and 7 μg mL^{-1} in each case. After a 24 h exposure, test cultures were evaluated for their ability to exclude the dye Trypan Blue. In CH 2983, HeLa and MG 63 cultures, control cells were always 98–100% viable (±4%). In all cases, there was some evidence for increased cell detachment during washing and staining, in the wells exposed to the highest concentrations of compounds **1** and **22**. In the case of CH 2983 and MG 63, the treated cells retained their ability to exclude dye to the same extent as the controls. In the case of the HeLa cells, viability was 85% (±4%) at the highest concentrations and 91% (±4%) at the lowest.

We conclude that compounds **1** and **22** are not significantly toxic to non-proliferating cells at concentrations below those which result in inhibition of proliferation, and that the MIC's against multidrug resistant Gram-positive bacteria are lower (sevenfold in the case of totarol) than the minimum concentration at which cytotoxicity is evident against proliferating human cell lines. Further testing would be required to determine if this window of safety is sufficient to permit the use of these compounds in the treatment of human bacterial disease.

Experimental

Synthesis

General methods. ^{1}H and ^{13}C NMR spectroscopic data were recorded on a Bruker AC300 spectrometer and assignments were made with the assistance of DEPT and COSY experiments. Sugar resonances are identified by primes (′). The infrared spectra were obtained (KBr) on a Perkin–Elmer 1600 series FTIR spectrometer. Elemental analyses were conducted with a Carlo Erba EA 1108 elemental analyser. Low and high resolution

mass spectra (HRMS) were obtained on a VG-70-250S double focusing magnetic sector mass spectrometer (VG Analytical) equipped with a standard VG-70S EI/CI ion source. Melting points were determined on a Reichert hot stage microscope and are uncorrected. All reactions were monitored by thin layer chromatography which was carried out on 60 PF254 silica gel-coated aluminum sheets. All purifications were by column chromatography and were performed using Merck Kieselgel S silica gel. Solvents were dried and purified before use according to standard procedures.[44] "Petrol" refers to the fraction of petroleum ether boiling between 60 and 80°C.

Podocarpa-8,11,13-trien-13-ol (2).[21] A solution of enone **2a**[19,20] (110 mg, 0.45 mmol) and *N*-bromosuccinimide (90 mg, 0.5 mmol) in carbon tetrachloride (10 mL) was heated at reflux by irradiation under a heat lamp for 1 h and then cooled, diluted with dichloromethane (50 mL) and filtered through Celite. The resulting solution was washed with $NaHCO_3$ (2×20 mL), brine (2×20 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude residue was redissolved in collidine (5 mL) and heated under reflux for 30 min. The mixture was diluted with HCl (100 mL, 1 M), extracted with ethyl acetate (3×25 mL), and the extract was dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (10/1) as the eluant gave compound **2** (84 mg, 75%), mp 128°C, lit.[21] 127–127.5°C; ^{1}H NMR ($CDCl_3$) δ 7.03 (1H, d, J=8.5 Hz, H-11), 6.53 (1H, dd, J=8.5, 2.8 Hz, H-12), 6.42 (1H, d, J=2.8 Hz, H-14), 4.68 (1H, br s, OH), 2.76 (2H, m, H-7), 1.08 (3H, s, H-20), 0.87 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.8, 142.8, 136.8, 125.6, 114.8, 112.8 (Ar), 50.5 (C-5), 41.7 (C-3), 39.0 (C-1), 37.2 (C-10), 33.4 (C-4), 33.2 (C-18), 30.4 (C-7), 24.9 (C-20), 21.5 (C-19), 19.3 (C-6), 18.3 (C-2).

14-Methylpodocarp-8(14)-en-13-one (3a). Methyl iodide (88 μL, 1.4 mmol) in anhydrous *tert*-butanol (5 mL) was added dropwise to a stirred solution of compound **2a** (318 mg, 1.3 mmol) and potassium *tert*-butoxide (174 mg, 1.6 mmol) in *tert*-butanol (10 mL) under reflux over a 15 min period. After an additional 15 min under reflux the reaction was quenched with aqueous ammonium chloride (150 mL, 10% w/v) and the resulting aqueous layer extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (12/1) as the eluant gave **3a** as a chromatographically pure oil (152 mg, 45%); IR υ_{max} 1667 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$) δ 2.95 (1H, ddd, J=16.0, 4.9, 1.9 Hz, H-7β), 2.43 (1H, dt, J=15.5, 4.2, 4.2 Hz, H-12a), 2.18 (1H, dd, J=15.5, 5.4 Hz, H-12b), 1.79 (3H, s, CH_3), 0.93 (3H, s, H-18), 0.87 (3H, s, H-19), 0.76 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) δ 199.6 (C-13), 158.0 (C-8), 130.5 (C-14), 53.8 (C-5), 52.7 (C-9), 41.9 (C-3), 39.1 (C-10), 39.1 (C-1), 37.0 (C-12), 33.6 (C-18), 33.4 (C-4), 31.3 (C-7), 22.1 (C-19), 21.8 (C-6), 20.3 (C-11), 18.9 (C-2), 15.2 (C-20), 11.1 (CH_3); m/z 260 (M^+, 14%), 149 (19), 137 (54), 124 (100), 95 (14), 69 (14); HRMS, calcd for $C_{18}H_{28}O$ (M^+) 260.2140, found 260.2137.

14-Ethylpodocarp-8(14)-en-13-one (4a).[24] Bromoethane (71 μL, 0.96 mmol) in anhydrous *tert*-butanol (5 mL) was added dropwise to a stirred solution of compound **2a** (223 mg, 0.91 mmol) and potassium *tert*-butoxide (122 mg, 1.1 mmol) in *tert*-butanol (10 mL) under reflux conditions over a 15 min period. After an additional 15 min under reflux the reaction was quenched with aqueous ammonium chloride (150 mL, 10% w/v) and the resulting aqueous layer extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (12/1) as the eluant gave **4a** as a chromatographically pure oil (140 mg, 56%); ^{1}H NMR ($CDCl_3$) δ 2.95 (1H, ddd, J=15.5, 4.7, 2.0 Hz, H-7β) 2.30 (2H, q, J=7.6 Hz, CH_2), 0.93 (3H, s, H-18), 0.90 (3H, t, J=7.6 Hz, CH_3), 0.88 (3H, s, H-19), 0.76 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) δ 199.0 (C-13), 157.4 (C-8), 136.7 (C-14), 54.0 (C-5), 52.3 (C-9), 41.8 (C-3), 39.3 (C-10), 39.0 (C-1), 37.1 (C-12), 33.5 (C-18), 33.3 (C-4), 30.9 (C-7), 21.1 (C-6), 22.0 (C-19), 20.0 (CH_2), 18.8 (C-11), 18.5 (C-2), 14.9 (C-20), 13.9 (CH_3); HRMS, calcd for $C_{19}H_{30}O$ (M^+) 274.2297, found 274.2298.

14-Propylpodocarp-8(14)-en-13-one (5a). 1-Bromopropane (124 μL, 1.4 mmol) in anhydrous *tert*-butanol (5 mL) was added dropwise to a stirred solution of compound **2a** (318 mg, 1.3 mmol) and potassium *tert*-butoxide (174 mg, 1.6 mmol) in *tert*-butanol alcohol (10 mL) under reflux over a 15 min period. After an additional 15 min under reflux the solution was quenched with aqueous ammonium chloride (150 mL, 10% w/v) and the resulting aqueous layer extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (12/1) as the eluant gave **5a** as an oil (120 mg, 32%); IR υ_{max} 1668 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$) δ 2.96 (1H, ddd, J=16.0, 4.9, 1.9 Hz, H-7β), 2.30 (2H, q, J=7.4 Hz, CH_2CH_3), 0.93 (3H, s, H-18), 0.89 (3H, t, J=7.4 Hz, CH_3), 0.87 (3H, s, H-19), 0.76 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) δ 199.5 (C-13), 157.8 (C-8), 135.4 (C-14), 54.2 (C-5), 52.6 (C-9), 42.0 (C-3), 39.5 (C-10), 39.1 (C-1), 37.2 (C-12), 33.7 (C-18), 33.5 (C-4), 31.4 (C-7), 27.4 (CH_2), 22.9 (CH_2), 22.3 (C-6), 22.1 (C-19), 20.1 (C-11), 19.0 (C-2), 15.1 (C-20), 14.2 (CH_3); m/z 288 (M^+, 100%), 165 (24), 137 (74), 123 (27), 91 (23), 81 (24), 69 (22); HRMS, calcd for $C_{20}H_{32}O$ (M^+) 288.4453, found 288.4571.

14-Methylpodocarpa-8,11,13-trien-13-ol (3). Copper(II) bromide (62 mg, 0.28 mmol) was added to a stirred solution of compound **3a** (60 mg, 0.23 mmol) in acetonitrile (5 mL) at room temperature. After 6 h the reaction was quenched with saturated brine (100 mL) and the resulting aqueous solution extracted with ethyl acetate (3×30 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (20/1) as the eluant gave **3** (42 mg, 71%), mp 142°C; IR υ_{max} 3419 (OH), 1640 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.03, 6.64 (2H, 2d, J=8.5 Hz, H-11, H-12), 4.59 (1H, s, OH), 2.83 (1H, dd, J=16.5, 6.7 Hz, H-7β), 2.65 (1H, ddd, J=16.5,

11.3, 7.8 Hz, H-7α), 2.12 (3H, s, CH_3), 1.19 (3H, s, H-20), 0.98 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 150.8, 143.2, 135.4, 122.5, 121.4 112.7 (Ar), 49.9 (C-5), 41.7 (C-3), 39.5 (C-1), 37.5 (C-10), 33.4 (C-4), 33.3 (C-18), 28.8 (C-7), 25.1 (C-20), 21.6 (C-19), 19.5 (C-6), 19.1 (C-2), 11.1 (CH_3); *m/z* 258 (M^+, 34%), 243 (100), 173 (32), 161 (26), 147 (40), 69 (11); HRMS calcd for $C_{18}H_{26}O$ (M^+) 258.1984, found 258.1985.

14-Ethylpodocarpa-8,11,13-trien-13-ol (4). Copper(II) bromide (54 mg, 0.24 mmol) was added to a stirred solution of compound **4a** (54 mg, 0.20 mmol) in acetonitrile (5 mL) at room temperature. After 6 h the mixture was quenched with saturated brine solution (100 mL) and the resulting aqueous solution extracted with ethyl acetate (3×30 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (20/1) as the eluant gave **4** as a chromatographically pure oil (35 mg, 64%); IR υ_{max} 3443 (OH), 1652 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.03, 6.62 (2H, 2d *J* = 8.6 Hz, H-11, H-12), 4.57 (1H, s, OH), 2.96 (1H, dd, *J* = 16.1, 6.9 Hz, H-7α), 2.76 (1H, ddd, *J* = 16.1, 11.2, 7.9 Hz, H-7β), 2.65 (2H, q, *J* = 7.6 Hz, CH_2), 1.20 (3H, s, H-20), 1.16 (3H, t, *J* = 7.6 Hz, CH_3), 0.98 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 150.6, 143.2, 134.3, 127.4, 122.7 112.8 (Ar), 49.8 (C-5), 41.6 (C-3), 39.3 (C-1), 37.5 (C-10), 33.2 (C-4), 33.2 (C-18), 27.5 (C-7), 25.0 (C-20), 21.5 (C-19), 19.3 (C-6), 19.0 (C-2), 18.9 (CH_2), 13.0 (CH_3); *m/z* 272 (M^+, 36%), 257 (100), 187 (38), 175 (24), 161 (44), 69 (14); HRMS, calcd for $C_{19}H_{28}O$ (M^+) 272.2140, found 272.2142.

14-Propylpodocarpa-8,11,13-trien-13-ol (5). Copper(II) bromide (84 mg, 0.36 mmol) was added to a stirred solution of compound **5a** (90 mg, 0.30 mmol) in acetonitrile (5 mL) at room temperature. After 6 h the mixture was quenched with brine (100 mL) and the resulting aqueous solution extracted with ethyl acetate (3×30 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (20/1) as the eluant gave **5** as a chromatographically pure oil (72 mg, 80%); IR υ_{max} 3441 (OH), 1650, 1588 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) 7.02, 6.62 (2H, 2d *J* = 8.6 Hz, H-11, H-12), 4.59 (1H, s, OH), 2.75 (2H, m, H-7α, H-7β), 1.19 (3H, s, H-20), 1.04 (3H, t, *J* = 7.3 Hz, CH_3), 0.97 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 151.0, 143.4, 134.8, 126.3, 123.0 113.0 (Ar), 50.1 (C-5), 41.8 (C-3), 39.6 (C-1), 37.8 (C-10), 33.5 (C-4), 33.4 (C-18), 28.3 (C-7), 28.0 (CH_2), 25.3 (C-20), 22.3 (CH_2), 21.7 (C-19), 19.6 (C-6), 19.3 (C-2), 14.9 (CH_3): *m/z* 286 (M^+, 41%), 272 (24), 271 (100), 201 (34), 189 (18), 175 (40); HRMS, calcd for $C_{20}H_{30}O$ (M^+) 286.2297, found 286.2294.

7α-Acetoxy-13-methoxytotara-8,11,13-triene (6a) and 14-acetoxy-13-methoxypodocarpa-8,11,13-triene (6b). Lead tetraacetate (1.7 g, 3.8 mmol) was added to a stirred solution of **11** (1 g, 3.3 mmol) in acetic acid (10 mL) and the mixture was heated to 100°C under argon. It was then stirred for 2 h, another portion of lead tetraacetate (0.85 g, 1.9 mmol) was added and the solution was stirred for a further 2 h. The mixture was diluted with water (200 mL) and extracted with ethyl acetate (3×50 mL), and the combined organic extracts were washed with water until the washings were neutral, dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (40/1) as the eluant gave: 7-acetoxy-13-methoxytotara-8,11,13-triene (**6a**) (502 mg, 42%), mp 96–97°C; ^1H NMR ($CDCl_3$) δ 7.16, 6.87 (2H, 2d, *J* = 8.8 Hz, H-11, H-12), 6.10 (1H, br t, H-7β), 3.78 (3H, s, OCH_3), 3.26 (1H, br s, H-15), 2.90 (2H, m, H-6), 2.04 (3H, s, $COCH_3$), 1.32, 1.28 (2×3H, 2d, *J* = 7.0 Hz, H-16, H-17), 1.15 (3H, s, H-20), 0.90 (3H, s, H-18), 0.89 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 170.2 (*C*OCH_3), 156.8, 143.7, 135.5, 130.8, 123.3, 112.8 (Ar), 69.4 (C-7), 55.1 (OCH_3), 44.5 (C-5), 41.2 (C-3), 39.1 (C-1), 38.1 (C-10), 33.2 (C-4), 32.7 (C-18), 28.3 (C-15), 26.6 (C-6), 24.5 (C-20), 21.4 (C-19), 20.7 (CO*C*H_3), 20.4, 20.3 (C-16, C-17), 19.4 (C-2); *m/z* 358 (M^+, 7%), 298 (100), 241 (54), 213 (46), 189 (30), 171 (41), 83 (13), 69 (22); HRMS, calcd for $C_{23}H_{34}O_3$ (M^+) 358.2508, found 358.2506; and 14-acetoxy-13-methoxypodocarpa-8,11,13-triene (**6b**) as a chromatographically pure oil (163 mg, 16%); IR υ_{max} 3455 (OH), 1737 (C=O), 1641 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.08, 6.77 (2H, 2d, *J* = 8.7 Hz, H-11, H-12), 3.77 (3H, s, OCH_3), 2.77 (1H, dd *J* = 17.6, 6.5 Hz, H-7β), 2.55 (1H, ddd, *J* = 17.6, 11.3, 7.9 Hz, H-7α) 2.31 (3H, s, $COCH_3$), 1.16 (3H, s, H-20), 0.93 (3H, s, H-18), 0.91 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 168.9 (*C*OCH_3), 148.3, 143.8, 137.4, 129.2, 122.1, 109.7 (Ar), 55.9 (OCH_3), 49.8 (C-5), 41.7 (C-3), 39.1 (C-1), 37.4 (C-10), 33.4 (C-4), 33.3 (C-18), 25.1 (C-20), 24.5 (C-7), 21.6 (C-19), 20.5 (CO*C*H_3), 19.3 (C-6), 18.2 (C-2); *m/z* 316 (M^+, 15%), 299 (65), 281 (49), 257 (45), 43 (100); HRMS, calcd for $C_{20}H_{28}O_3$ (M^+) 316.4405, found 316.4411.

13-Methoxypodocarpa-8,11,13-trien-14-ol (6c). Sodium hydride (20 mg) was added to a stirred solution of **6b** (2.2 g, 7.0 mmol) in methanol (20 mL) at room temperature under argon. Stirring was continued for 14 h and the mixture was concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (20/1) as the eluant gave compound **6c** (1.42 g, 75%), mp 97°C; IR υ_{max} 3442 (OH), 1618 (C=C), 1491 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.75, 6.69 (2H, dd, *J* = 8.6 Hz, H-11, H-12), 5.62 (1H, s, OH), 3.84 (3H, s, OCH_3), 2.93 (1H, dd, *J* = 17.9, 6.1 Hz, H-7β), 2.66 (1H, ddd, *J* = 17.9, 11.4, 8.0 Hz, H-7α), 1.17 (3H, s, H-20), 0.95 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 144.2, 143.1, 142.3, 122.1, 114.7, 107.9 (Ar), 55.8 (OCH_3), 49.9 (C-5), 41.6 (C-3), 39.0 (C-1), 37.2 (C-10), 33.2 (C-4), 33.2 (C-18), 24.8 (C-20), 24.0 (C-7), 21.5 (C-19), 19.2 (C-6), 18.2 (C-2); *m/z* 274 (M^+, 98%), 259 (100), 189 (38), 163 (43), 115 (10), 69 (28); HRMS, calcd for $C_{18}H_{26}O_2$ (M^+) 274.1933, found 274.1932.

Podocarpa-8,11,13-trien-13,14-diol (6).[21] Boron tribromide (1 M, 1 mL) was added dropwise to a stirred solution of **6c** (100 mg, 0.36 mmol) in CH_2Cl_2 (10 mL) at room temperature under argon. The mixture was stirred for 30 min, diluted with CH_2Cl_2 (50 mL), washed with aqueous $Na_2S_2O_3$ (10%, 3×25 mL), aqueous $NaHCO_3$ (3×25 mL), brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using

petrol/ethyl acetate (9/1) as the eluant gave **6** (78 mg, 83%), mp 158–159°C, lit.[21] 146.5–147.5°C; ^{1}H NMR ($CDCl_3$) δ 6.74, 6.70 (2H, 2d, J = 8.5 Hz, H-11, H-12), 5.16 (2H, s, OH), 2.86 (1H, dd, J = 17.1, 6.6 Hz, H-7β), 2.63 (1H ddd, J = 17.1, 11.3, 7.9 Hz, H-7α), 1.18 (3H, s, H-20), 0.97 (3H, s, H-18), 0.94 (3H, s, H-19) (effectively identical to those recorded at 60 MHz[20]); ^{13}C NMR ($CDCl_3$) δ 144.1, 140.9, 140.0, 122.5, 116.0, 112.7 (Ar), 50.0 (C-5), 41.7 (C-3), 39.1 (C-1), 37.2 (C-10), 33.3 (C-4), 33.3 (C-18), 25.0 (C-20), 24.2 (C-7), 21.6 (C-19), 19.3 (C-6), 18.2 (C-2).

Totara-8,11,13-trien-13-yl trifluoromethanesulfonate (13). Trifluoromethanesulfonic anhydride (0.98 mL, 5.4 mmol) was added dropwise to a stirred solution of totarol (**1**) (1.05 g, 3.6 mmol) and triethylamine (1 mL, 7.2 mmol) in CH_2Cl_2 (20 mL) at 0°C under argon. The resulting solution was allowed to warm to room temperature, stirred for 14 h, diluted with CH_2Cl_2 (200 mL), washed with dilute HCl (2×50 mL, 1M), brine (3×50 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol as the eluant gave **13** (960 mg, 63%), mp 78°C; IR_{max} 1638 (C=C), 1211, 1142 (SO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.18, 7.08 (2H, 2d, J = 9.0 Hz, H-11, H-12), 2.97 (1H, dd, J = 17.2, 5.9 Hz, H-7β), 2.80 (1H, ddd, J = 17.2, 10.8, 7.9 Hz, H-7α), 1.36, 1.33 (2×3H, d, J = 7.1 Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.96 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 150.6, 137.1, 124.1, 120.7 (Ar), 118.5 (q, J = 320 Hz, CF_3), 118.3, 116.4 (Ar), 49.2 (C-5), 41.5 (C-3), 39.4 (C-1), 38.4 (C-10), 33.4 (C-4), 33.2 (C-18), 28.8 (C-7), 27.2 (C-15), 24.9 (C-20), 21.6 (C-19), 20 .8, 20.7 (C-16, C-17), 19.4 (C-6), 19.2 (C-2); m/z 418 (M^+, 46%), 403 (49), 321 (100), 307 (48), 161 (22), 84 (70), 69 (65); HRMS, calcd for $C_{21}H_{29}F_3O_3S$ (M^+) 418.1790, found 418.1788.

Totara-8,11,13-triene (7).[27] Palladium on carbon (10%, 20 mg) was suspended in a stirred solution of triethylamine (1.0 mL) and **13** (100 mg, 0.24 mmol) in methanol (30 mL) at room temperature under an atmosphere of hydrogen, and the mixture was stirred for 14 h, filtered through Celite and concentrated in vacuo. Chromatography on silica gel using petrol as the eluant gave **7** (62 mg, 96%), mp 78–79°C, lit.[27] 78–78.5°C; ^{1}H NMR ($CDCl_3$) δ 7.18–7.04 (3H, m, Ar) 3.13 (1H, sept, J = 6.8 Hz, H-15), 2.96, (1H, dd, J = 17.2, 6.8 Hz, H-7β), 2.78 (1H, ddd, J = 17.2, 11.2, 7.8 Hz, H-7α), 1.22, 1.21 (2×3H, d, J = 7.0 Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.95 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 150.3, 146.6, 132.1, 125.7, 122.1, 121.8 (Ar), 49.6 (C-5), 41.6 (C-3), 39.4 (C-1), 38.2 (C-10), 33.4 (C-4), 33.2 (C-18), 28.2 (C-7), 27.2 (C-15), 25.0 (C-20), 23.7, 23.3 (C-16, C-17), 21.6 (C-19), 19.5 (C-6), 19.2 (C-2).

Podocarpa-8,11,13-trien-12-yl trifluoromethanesulfonate (8b). Trifluoromethanesulfonic anhydride (80 μL, 0.44 mmol) was added dropwise to a stirred solution of **8a**[28] (100 mg, 0.41 mmol) and triethylamine (0.068 mL, 0.49 mmol) in CH_2Cl_2 (10 mL) at 0°C under argon. The resulting solution was allowed to warm to room temperature, stirred for 14 h, diluted with CH_2Cl_2 (100 mL), washed with dilute HCl (2×25 mL, 1M), brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol as the eluant gave **8b** as a chromatographically pure oil (100 mg, 65%); ^{1}H NMR ($CDCl_3$) δ 7.17–6.93 (3H, m, Ar), 2.99–2.71 (2H, m, H-7α,7β), 1.17 (3H, s, H-20), 0.96 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 153.1, 148.2, 136.0, 130.7 (Ar), 119.0 (q, J = 319 Hz, CF_3), 118.1, 117.5 (Ar), 50.0 (C-5), 41.9 (C-3), 38.8 (C-1), 38.5 (C-10), 33.9 (C-4), 33.6 (C-18), 30.0 (C-7), 25.0 (C-20), 21.9 (C-19), 19.3 (C-6), 18.8 (C-2); m/z 376 (M^+, 47%), 361 (53), 347 (46), 305 (22), 291 (57), 279 (100), 265 (71), 159 (18), 129 (18), 115 (20), 83 (22), 69 (100), 55 (28), 41 (28); HRMS, calcd for $C_{18}H_{23}F_3O_3S$ (M^+) 376.1320, found 376.1323.

Podocarpa-8,11,13-triene (8).[29] Palladium on carbon (10%, 20 mg) was suspended in a stirred solution of triethylamine (0.2 mL), methanol (10 mL) and **8b** (100 mg, 0.27 mmol) at room temperature under an atmosphere of hydrogen and the mixture was stirred for 14 h, filtered through Celite and concentrated in vacuo. Chromatography on silica gel using petrol as the eluant gave **8** as a chromatographically pure oil (55 mg, 91%); ^{1}H NMR ($CDCl_3$) δ 7.18–7.00 (4H, m, Ar), 2.93–2.87 (2H, m, H-7α,7β), 1.19 (3H, s, H-20), 0.95 (3H, s, H-18), 0.93 (3H, s, H-19) consistent with literature data[45]; ^{13}C NMR ($CDCl_3$) δ 150.1, 135.2, 128.9, 125.5, 125.1, 124.3 (Ar), 50.3 (C-5), 41.7 (C-3), 38.8 (C-1), 37.8 (C-10), 33.4 (C-4), 33.3 (C-18), 30.3 (C-7), 24.8 (C-20), 21.6 (C-19), 19.3 (C-6), 19.0 (C-2).

Abieta-8,11,13-triene-12-ol (10, ferruginol).[30] Sodium hydride (10 mg) was added to a stirred solution of ferruginyl benzoate (**9**, 163 mg, 0.42 mmol, prepared according to ref. 29) in methanol (10 mL) at room temperature under argon. The resulting solution was stirred for 14 h and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (19/1) as the eluant gave **10** (105 mg, 88%), mp 56–57°C, lit.[31] 56–57°C; ^{1}H NMR ($CDCl_3$) δ 6.82 (1H, s, H-14), 6.62 (1H, s, H-11), 3.13 (1H, sept J = 6.9 Hz, H-15), 2.90–2.70 (2H, H-7α,7β), 1.23, 1.22 (2×3H, d, J = 6.9 Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.95 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 150.6, 148.5, 132.8, 131.4, 126.5, 110.9 (Ar), 50.2 (C-5), 41.6 (C-3), 38.7 (C-1), 37.4 (C-10), 33.3 (C-4), 33.2 (C-18), 29.6 (C-7), 26.7 (C-15), 24.6 (C-20), 22.6, 22.4 (C-16, C-17), 21.5 (C-19), 19.2 (C-6), 19.1 (C-2).

13-Methoxytotara-8,11,13-triene (11).[7,36] Methyl iodide (0.23 mL, 3.7 mmol) was added to a stirred solution of sodium hydride (60%, 0.22 g, 5.5 mmol) and totarol (**1**, 1.04 g, 3.6 mmol) in DMF (10 mL) at 0°C under argon. The resulting suspension was allowed to warm to room temperature, stirred for 14 h, diluted with water (200 mL) and filtered to yield a solid. Chromatography on silica gel with petrol/ethyl acetate (100/1) as the eluant gave **11** (1.01, 93%); mp 92–93°C, lit.[36] 92–92.5°C; ^{1}H NMR ($CDCl_3$) δ 7.16, 6.79 (2H, 2d, J = 8.8 Hz, H-11, H-12), 3.83 (3H, s, OCH_3), 3.34 (1H, m, H-15), 3.02, (1H, dd, J = 17.0, 6.3 Hz, H-7β), 2.84 (1H, ddd, J = 17.0, 11.2, 7.8 Hz, H-7α), 1.37, 1.36 (2×3H, d, J = 7.0 Hz, H-16, H-17), 1.27 (3H, s, H-20),

1.02 (3H, s, H-18), 1.00 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 156.4, 143.1, 133.7, 133.4, 122.8, 109.6 (Ar), 55.1 (OCH_3), 49.7 (C-5), 41.7 (C-3), 39.7 (C-1), 37.8 (C-10), 33.3 (C-4), 33.3 (C-18), 28.6 (C-7), 27.4 (C-15), 25.3 (C-20), 21.7 (C-19), 20 .5, 20.5 (C-16, C-17), 19.6 (C-6), 19.5 (C-2).

13-Acetoxytotara-8,11,13-triene (12).[17,36] Acetic anhydride (20 mL) was added to a stirred solution of totarol (**1**, 8.6 g, 30 mmol) in pyridine (20 mL) at 0°C. The resulting solution was allowed to warm to room temperature, stirred for 14 h, diluted with water (400 mL) and filtered to yield a solid. Chromatography on silica gel using petrol/ethyl acetate (50/1) as the eluant gave **12** (7.8 g, 79%), mp 120°C, lit.[36] 121–121.5°C; ^{13}C NMR spectral chemical shifts identical to published data (δ ± 0.1 ppm).[17]

13-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-totara-8,11,13-triene (14). A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide[37] (431 mg, 1.1 mmol) in toluene (5 mL) was added dropwise to a stirred suspension of **1** (200 mg, 0.7 mmol), activated sieves (3 Å, 1.0 g), and sodium hydrogen phosphate (1.0 g) in toluene under argon in a flask masked from the light. After 30 min silver triflate (270 mg, 1.1 mmol) in toluene (5 mL) was added via a cannula, the mixture was stirred for 2 h, filtered through Celite and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (3/1) as the eluant gave **14** (270 mg, 63%), mp 175°C; IR υ_{max} 1746 (CO), 1640 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.07, 6.79 (2H, 2d, J = 8.9 Hz, H-11, H-12), 5.33 (1H, t, J = 9.1 Hz, H-2′), 5.30 (1H, t, J = 9.1 Hz, H-3′), 5.18 (1H, t, J = 9.1 Hz, H-4′), 5.18 (1H, d, J = 9.1 Hz, H-1′), 4.25 1H dd, J = 12.2, 5.6 Hz, H-6′a), 4.16 (1H, dd, J = 12.2, 2.6 Hz, H-6′b) 3.86 (1H, m, H-5′), 3.27 (1H, br s, H-15), 2.92, (1H, dd, J = 17.0, 6.1 Hz, H-7β), 2.76 (1H, ddd, J = 17.0, 10.9, 7.8 Hz, H-7α), 2.06, 2.04, 2.02, 2.00 (4×3H, 4s, $COCH_3$), 1.28, 1.19 (2×3H, d, J = 7.0 Hz, H-16, H-17), 1.18 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.3, 169.4, 169.3 ($COCH_3$), 153.0, 145.2, 134.4, 134.0, 122.9, 111.8 (Ar), 97.5 (C-1′), 73.4 (C-2′), 71.8 (C-3′), 71.4 (C-4′), 68.6 (C-5′), 62.2 (C-6′), 49.5 (C-5), 41.5 (C-3), 39.6 (C-1), 37.9 (C-10), 33.3 (C-4), 33.2 (C-18), 28.9 (C-7), 27.2 (C-15), 25.2 (C-20), 21.6 (C-19), 20.8, 20.7 ($COCH_3$), 20.6, 20.6 (C-16, C-17), 20.4, 20.4 ($COCH_3$), 19.5 (C-6), 19.4 (C-2); m/z 616 (M^+, 1%), 331 (42), 271 (13), 169 (100), 109 (38), 69 (6), 43 (38); HRMS, calcd for $C_{34}H_{48}O_{10}$ (M^+) 616.3247, found 616.3257.

13-(2,3,4,6-Tetra-*O*-acetyl-α- and β-D-galactopyranosyloxy)-totara-8,11,13-triene (15 and 16). A solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide[37] (431 mg, 1.1 mmol) in toluene (5 mL) was added dropwise to a stirred suspension of **1** (200 mg, 0.7 mmol), activated sieves (3 Å, 1 g), and sodium hydrogen phosphate (1 g) in toluene under argon in a flask masked from the light. After 30 min silver triflate (270 mg, 1.1 mmol) in toluene (5 mL) was added via a cannula, the mixture was stirred for 2 h, filtered through Celite and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (5.6/1) as the eluant gave: 13-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxy)-totara-8,11,13-triene (**15**) as a chromatographically pure oil (65 mg, 15%); ^{1}H NMR ($CDCl_3$) δ 7.08, 6.98 (2H, 2d, J = 9.0 Hz, H-11, H-12), 5.79 (1H, d, J = 3.2 Hz, H-1′), 5.69 (1H, 2d, J = 10.9, 3.2 Hz, H-2′), 5.55 (1H, d, J = 2.9 Hz, H-4′), 5.35 (1H, dd, J = 10.9, 3.6 Hz, H-3′), 4.27 (1H, t, J = 6.6 Hz, H-5′), 4.12 (2H, m, H-6′), 3.35 (1H, br s, H-15), 2.99 (1H, dd, J = 17.1, 6.3 Hz, H-7β), 2.75 (1H, m, H-7α), 2.18, 2.04, 2.01, 1.94 (4×3H, 4s, $COCH_3$), 1.39, 1.36 (2×3H, d, J = 7.0 Hz, H-16, H-17), 1.16 (3H, s, H-20), 0.95 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 170.2, 170.1, 170.0, 169.9 ($COCH_3$), 152.7, 144.9, 134.0, 133.5, 123.0, 111.6 (Ar), 93.9 (C-1′), 67.9 (C-3′), 67.8 (C-2′), 67.8 (C-4′), 66.9 (C-5′), 61.1 (C-6′), 49.3 (C-5), 41.5 (C-3), 39.5 (C-1), 37.7 (C-10), 33.2 (C-4), 33.1 (C-18), 28.7 (C-7), 27.3 (C-15), 25.0 (C-20), 21.5 (C-19), 20.9, 20.6, 20.5, 20.5 ($COCH_3$), 20.6, 20.6 (C-16, C-17), 19.3 (C-6), 19.2 (C-2); m/z 616 (M^+, 3%), 331 (100), 169 (94), 127 (13), 109 (39), 43 (97); HRMS, calcd for $C_{34}H_{48}O_{10}$ (M^+) 616.3247, found 616.3255. This was followed by 13-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-totara-8,11,13-triene (**16**) as a chromatographically pure oil (180 mg, 42%); ^{1}H NMR ($CDCl_3$) δ 7.08, 6.82 (2H, 2d, J = 8.9 Hz, H-11, H-12), 5.54 (1H, dd, J = 10.3, 8.0 Hz, H-2′), 5.45 (1H, d, J = 3.3 Hz, H-4′), 5.14 (1H, d, J = 8.0 Hz, H-1′), 5.11 (1H, dd, J = 10.3, 3.3 Hz, H-3′), 4.14 (3H, m, H-5′, 2H-6′), 3.29 (1H, br s, H-15), 2.93 (1H, dd, J = 17.1, 6.1 Hz, H-7β), 2.78 (1H, ddd, J = 17.1, 10.8, 7.8 Hz, H-7α), 2.20, 2.07, 2.02, 2.01 (4×3H, s, $COCH_3$), 1.32, 1.21 (2×3H, d, J = 7.1 Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.96 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.4, 170.2, 169.3 ($COCH_3$), 153.2, 145.2, 134.4, 134.0, 122.9, 112.0 (Ar), 98.2 (C-1′), 71.5 (C-3′), 70.9 (C-5′), 68.8 (C-2′), 67.2 (C-4′), 61.5 (C-6′), 49.6 (C-5), 41.6 (C-3), 39.6 (C-1), 37.9 (C-10), 33.4 (C-4), 33.3 (C-18), 29.0 (C-7), 27.3 (C-15), 25.3 (C-20), 21.7 (C-19), 21.0, 20.8, 20.7, 20.6 ($COCH_3$), 20.6, 20.5 (C-16, C-17), 19.5 (C-6), 19.4 (C-2); m/z 616 (M^+, 3%), 331 (83), 169 (78), 127 (13), 109 (34), 43 (100); HRMS, calcd for $C_{34}H_{48}O_{10}$ (M^+) 616.3247, found 616.3257.

13-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-totara-8,11,13-triene (17). A solution of glycosyl donor tetra-*O*-acetyl-α-D-mannopyranosyl bromide[37] (470 mg, 1.2 mmol) in toluene (5 mL) was added dropwise to a stirred suspension of **1** (200 mg, 0.7 mmol), activated sieves (3 Å, 1 g), and sodium hydrogen phosphate (1 g) in toluene under argon in a flask masked from the light. After 30 min silver triflate (270 mg, 1.1 mmol) in toluene (5 mL) was added via cannula, the mixture was stirred for 2 h, filtered through Celite and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (5.6/1) as the eluant gave **17** (320 mg, 74%), mp 205°C; ^{1}H NMR ($CDCl_3$) δ 7.08 (2H, 2d, J = 8.9 Hz, H-11, H-12), 5.48 (2H, m, H-1′, H-2′), 5.59 (1H, dd, J = 10.0, 2.6 Hz, H-3′), 5.42 (1H, t, J = 10.0 Hz, H-4′), 4.33 (1H, dd, J = 12.8, 5.7 Hz, H-6′a), 4.11 (2H, m, H-5′, H-6′b), 3.34 (1H, sept J = 7.0 Hz, H-15), 2.96 (1H, dd, J = 17.1, 6.2 Hz, H-7β), 2.77 (1H, ddd, J = 17.1, 11.1, 7.9 Hz, H-7α) 2.19, 2.08, 2.07, 2.04 (4×3H, 4s, $COCH_3$), 1.42, 1.35 (2×3H, d, J = 7.0 Hz, H-16, H-17), 1.20 (3H, s, H-20), 0.96 (3H, 4s, H-18), 0.89 (3H, s, H-19); ^{13}C

NMR ($CDCl_3$) δ 170.4, 169.8, 169.8, 169.6 ($COCH_3$), 152.8, 145.2, 134.1, 133.7, 123.1, 112.1 (Ar), 95.9 (C-1′), 69.3 (C-2′), 69.1 (C-3′), 69.1 (C-5′), 65.6 (C-4′), 62.1 (C-6′), 49.3 (C-5), 41.4 (C-3), 39.5 (C-1), 37.6 (C-10), 33.1 (C-4), 33.0 (C-18), 28.6 (C-7), 27.1 (C-15), 25.0 (C-20), 21.4 (C-19), 20.9, 20.8, 20.7, 20.5 ($COCH_3$), 20.5, 20.5 (C-16, C-17), 19.3 (C-6), 19.2 (C-2); *m/z* 616 (M^+, 2%), 331 (53), 271 (10), 169 (97), 127 (12), 109 (41), 43 (100); anal., calcd for $C_{34}H_{48}O_{10}$ C, 66.2; H, 7.8, found C, 66.1; H, 8.0.

13-(2,2′,3,3′,4,6,6′-Hepta-*O*-acetyl-β-D-lactopyranosyloxy)-totara-8,11,13-triene (18). A solution of hepta-*O*-acetyl-α-D-lactopyranosyl bromide[37] (750 mg, 1.1 mmol) in toluene (5 mL) was added dropwise to a stirred suspension of **1** (286 mg, 1 mmol), activated sieves (3 Å, 1 g), and sodium hydrogen phosphate (1 g) in toluene under argon in a flask masked from the light. After 30 min silver triflate (386 mg, 1.5 mmol) in toluene (5 mL) was added via a cannula, the mixture was stirred for 2 h, filtered through Celite and concentrated in vacuo. Chromatography on silica gel using petrol/ethyl acetate (5.6/1) as the eluant gave **18** as a chromatographically pure oil (260 mg, 29%); ^{1}H NMR (DMSO-d_6) δ 7.05, 6.78 (2H, 2d, J = 8.9 Hz, H-11, H-12), 5.36–3.77 (13H, m, H-2′–H-6′, H-1″-H-6″), 4.51 (1H, d, J = 7.9 Hz, H-1′), 2.84 (2H, m, H-7a,7b), 2.16, 2.07, 2.07, 2.06, 2.06, 2.01, 1.97 (7×3H, 5s, $COCH_3$), 1.26, 1.16 (2×3H, d, J = 6.9 Hz, H-16, H-17), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); ^{13}C NMR (DMSO-d_6) δ 170.4, 170.2, 170.1, 169.9, 169.9 169.8, 169.6, 169.1 ($COCH_3$), 153.0, 145.0, 135.0, 133.8, 122.9 (Ar), 101.2 (C-1′), 97.0 (C-1″) 76.5, 73.5, 72.6, 71.7, 71.0, 70.8, 69.2, 66.7 (C-2′–C-5′, C-2″–C-5″), 62.2, 60.9 (C-6′, C-6″), 49.6 (C-5), 41.6 (C-3), 39.6 (C-1), 37.9 (C-10), 33.3 (C-4), 33.2 (C-18), 28.9 (C-7), 27.0 (C-15), 25.2 (C-20), 21.6 (C-19), 20.8, 20.8, 20.8, 20.7, 20.7, 20.7, 20.5 ($COCH_3$), 20.5, 20.4 (C-16, C-17), 19.5 (C-6), 19.4 (C-2); *m/z* 905 (MH^+, 3%), 619 (27), 559 (13), 331 (80), 169 (57); HRMS, calcd for $C_{46}H_{65}O_{18}$ $(MH)^+$ 905.4171, found 905.4158.

13-(β-D-Glucopyranosyloxy)-totara-8,11,13-triene (19). Sodium hydride (10 mg) was added to a stirred solution of **14** (90 mg, 0.15 mmol) in methanol (5 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. The resulting residue was suspended in 20% methanol in ethyl acetate and passed through a short silica gel column to afford **19** (61 mg, 93%), mp 128°C; IR υ_{max} 3374 (OH), 1646 (C=C) cm^{-1}; ^{1}H NMR (d_4-MeOH) δ 6.85, 6.72 (2H, 2d, J = 9.0 Hz, H-11, H-12), 4.75 (1H, d, J = 7.2 Hz, H-1′), 3.68 (1H, dd, J = 12.0, 1.5 Hz, H-6′a), 3.49 (1H, dd, J = 12.0, 5.0 Hz, H-6′b), 3.50–3.18 (4H, m, H-2′–H-5′), 3.11 (1H, br s, H-15), 2.74, (1H, dd, J = 16.9, 6.1 Hz, H-7β), 2.54 (1H, ddd, J = 16.9, 11.3, 7.8 Hz, H-7α), 1.17, 1.12 (2×3H, d, J = 7.0 Hz, H-16, H-17), 0.96 (3H, s, H-20), 0.76 (3H, s, H-18), 0.73 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 155.3, 145.4, 134.7, 130.0, 124.0, 113.5 (Ar), 78.9, 78.0, 75.2, 71.5, 68.6 (C-2′–C-5′), 62.7 (C-6′), 51.3 (C-5), 42.8 (C-3), 41.0 (C-1), 39.0 (C-10), 34.3 (C-4), 33.8 (C-18), 30.0 (C-7), 28.8 (C-15), 25.6 (C-20), 22.1 (C-19), 21.2, 21.2 (C-16, C-17), 20.6 (C-6), 20.6 (C-2); *m/z* 616 (M^+, 1%), 448 (2), 286 (62), 271 (100), 201 (26), 189 (12), 175 (36), 69 (15), 55 (10), 43 (19); HMRS, calcd for $C_{26}H_{40}O_6$ (M^+) 448.2825, found 448.2818.

13-(α-D-Galactopyranosyloxy)-totara-8,11,13-triene (20). Sodium hydride (10 mg) was added to a stirred solution of **15** (60 mg, 0.1 mmol) in methanol (10 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. The resulting residue was suspended in ethyl acetate/methanol (4/1) and passed through a short silica gel column to afford **20** (39 mg, 89%), mp 219°C; ^{1}H NMR (DMSO-d_6) δ 6.84, 6.78 (2H, 2d, J = 9.0 Hz, H-11, H-12), 5.19 (1H, d, J = 2.6 Hz, H-1′), 3.70–3.18 (6H, m, H-2′–H-6′), 2.72 (1H, dd, J = 16.8, 5.8 Hz, H-7′), 2.48 (1H, m, H-7′), 1.15, 1.11 (2×3H, d, J = 7.0 Hz, H-16, H-17), 0.95 (3H, s, H-20), 0.74 (3H, s, H-18), 0.70 (3H, s, H-19); ^{13}C NMR (DMSO-d_6) δ 155.2, 144.4, 134.0, 133.9, 124.3, 113.2 (Ar), 98.6 (C-1′), 73.5, 71.3, 69.7, 69.6 (C-2′–C-5′), 61.5 (C-6′), 50.8 (C-5), 42.6 (C-3), 40.7 (C-1), 38.7 (C-10), 34.5 (C-4), 34.4 (C-18), 29.8 (C-7), 28.6 (C-15), 26.5 (C-20), 22.9 (C-19), 22.1, 21.7 (C-16, C-17), 20.5 (C-6), 20.4 (C-2); *m/z* 449 (MH^+, 15%), 413 (13), 286 (100), 271 (94), 201 (24), 189 (30), 175 (26), 149 (21), 115 (19), 93 (36), 85 (15), 69 (41), 57 (17), 41 (35); HRMS, calcd for $C_{26}H_{40}O_6$ (M^+) 448.2825, found 448.2810.

13-(β-D-Galactopyranosyloxy)-totara-8,11,13-triene (21). Sodium hydride (20 mg) was added to a stirred solution of **16** (120 mg, 0.2 mmol) in methanol (10 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. The resulting residue was suspended in ethyl acetate/methanol (4/1) and passed through a short silica gel column to afford **21** (80 mg, 89%), mp 225°C; ^{1}H NMR (DMSO-d_6) δ 7.01, 6.88 (2H, 2d, J = 8.9 Hz, H-11, H-12), 4.74 (1H, d, J = 7.7 Hz, H-1′), 4.00–3.00 (7H, m, H-2′–H-6′, H-15), 2.60 (2H, m, H-7α,7β), 1.13, 1.09 (2×3H, d, J = 7.0 Hz, H-16, H-17), 0.93 (3H, s, H-20), 0.75 (3H, s, H-18), 0.71 (3H, s, H-19); ^{13}C NMR (DMSO-d_6) δ 155.4, 144.3, 134.0, 133.8, 123.9, 113.8 (Ar), 102.0 (C-1′), 76.7, 75.2, 72.0, 69.4 (C-2′–C-5′), 61.7 (C-6′), 50.6 (C-5), 42.4 (C-3), 40.4 (C-1), 38.6 (C-10), 34.3 (C-4), 34.2 (C-18), 29.6 (C-7), 28.2 (C-15), 26.3 (C-20), 22.7 (C-19), 22.0, 21.7 (C-16, C-17), 20.3 (C-6), 20.3 (C-2); *m/z* 449 (MH^+, 15%), 286 (100), 271 (66), 255 (18), 201 (23), 175 (16), 149 (17), 91 (22), 69 (29), 45 (25); HRMS, calcd for $C_{26}H_{41}O_6$ $(MH)^+$ 449.2903, found 449.2891.

13-(α-D-Mannopyranosyloxy)-totara-8,11,13-triene (22). Sodium hydride (20 mg) was added to a stirred solution of **17** (130 mg, 0.2 mmol) in methanol (10 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. The resulting residue was suspended in ethyl acetate/methanol (4/1) and passed through a short silica gel column to afford **22** (85 mg, 95%), mp 285°C; ^{1}H NMR (DMSO-d_6) δ 7.01, 6.88 (2H, 2d, J = 8.9 Hz, H-11, H-12), 4.56 (1H, d, J = 7.7 Hz, H-1′), 3.53–2.99 (7H, m, H2′–H6′, H-15), 2.61 (2H, m, H-7α,7β), 1.13, 1.09 (2×3H, d, J = 7.0 Hz, H-16, H-17), 0.93 (3H, s, H-20), 0.75 (3H, s, H-18), 0.71 (3H, s, H-19); ^{13}C NMR (DMSO-d_6) δ 155.4, 144.4, 134.0, 133.8, 123.9, 113.8 (Ar), 102.0 (C-1′), 76.7, 75.2,

72.0, 69.4 (C-2′–C-5′), 61.7 (C-6′), 50.6 (C-5), 42.4 (C-3), 40.4 (C-1), 38.6 (C-10), 34.3 (C-4), 34.2 (C-18), 29.6 (C-7), 28.2 (C-15), 26.3 (C-20), 22.7 (C-19), 22.0, 21.7 (C-16, C-17), 20.3 (C-6), 20.3 (C-2); *m/z* 448 (M^+, 17%), 307 (46), 286 (100), 271 (63), 189 (25), 175 (47); HRMS, calcd for $C_{26}H_{40}O_6Na$ $(MNa)^+$ 471.2723, found 471.2717.

13-(β-D-Lactopyranosyloxy)-totara-8,11,13-triene (23). Sodium hydride (20 mg) was added to a stirred solution of **18** (135 mg, 0.15 mmol) in methanol (10 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. The resulting residue was suspended in ethyl acetate/methanol (4/1) and passed through a short silica gel column to afford **23** (58 mg, 63%) mp 187°C; ^{1}H NMR (DMSO-d_6) δ 6.83, 6.69 (2H 2d, J = 8.8 Hz, H-11, H-12), 4.67 (1H, d, J = 7.7 Hz, H-1′), 4.06 (1H, d, J = 6.5 Hz, H1″), 3.60–3.10 (13H, m, H-2′–H-6′, H-2″–H-6″, H-15), 2.59 (2H, m, H-7), 1.12, 1.07 (2×3H, d, J = 6.9 Hz, H-16, H-17), 0.92 (3H, s, H-20), 0.74 (3H, s, H-18), 0.69 (3H, s, H-19); ^{13}C NMR (DMSO-d_6) δ 155.2, 144.6, 134.0, 133.9, 124.0, 113.7 (Ar), 105.1 (C-1″), 101.0 (C-1′), 81.6, 76.9, 76.8, 76.2, 74.7, 74.6, 71.9, 69.5 (C-2′–C-5′, C-2″–C-5″), 61.8, 61.7 (C-6′, C-6″), 50.7 (C-5), 42.4 (C-3), 40.5 (C-1), 38.6 (C-10), 34.3 (C-4), 34.2 (C-18), 29.6 (C-7), 27.5 (C-15), 26.3 (C-20), 22.7 (C-19), 21.9, 21.7 (C-16, C-17), 20.4 (C-6), 20.3 (C-2); *m/z* 610 (M^+, 3%), 271 (43), 189 (19), 165 (24), 115 (22), 91 (38), 41 (55); HRMS, calcd for $C_{32}H_{50}O_{11}$ (M^+) 610.3353, found 610.3351.

In vitro antibacterial screens

Minimum inhibitory concentrations (MIC) were determined by the double agar dilution method with Mueller–Hinton agar. The overnight broth cultures were diluted to approximately 10^8 CFU (colony forming units) mL^{-1} with fresh broth and an inoculum of 10^4 CFU mL^{-1} per spot was applied to agar plates containing graded concentrations of each compound. After incubation at 37°C for 18–20 h, the MIC was defined as the minimum drug concentration which inhibited growth of bacteria.

In vivo antibacterial screens

A mouse protection model was used to assess the in vivo antibacterial activity of compounds **1**, **22** and **23**. Mice were obtained from ZENECA Barriered Animal Breeding Unit. Male mice (Alpk: ApfCD-1 strain) weighing 19–21 g were infected intraperitoneally with ~ten times the LD_{50} of MSSA strain 601055 in 6% hog gastric mucin (Sigma). Animals were dosed subcutaneously with the test compounds at 1 and 5 h post infection. A group of four mice was used for each dose level of each compound. Animal health was monitored at least twice-daily until the experiment was terminated on day 4 post-infection. The dose of test compound that provided 50% protection (PD_{50} values) were computed by logit analysis from numbers of survivors over the range of doses tested.

Human cell culture screens

Conditions for the maintenance, culture and spectrophotometric measurement of growth in human cell cultures were as previously described.[46,47] Human fibroblasts were a normal diploid strain, CH 2983, established from a human amniocentesis sample; test cultures were all between the 6th and 10th passage. Human tumour cell lines were the HeLa carcinoma line, and an osteosarcoma line, MG 63.

Test cultures were plated sparsely in 0.1 mL aliquots in 96 well culture plates, in normal minimal Eagle's medium supplemented with 10% foetal calf serum. After 24 h, media were replaced with fresh 0.1 mL aliquots of media (control), or 0.1 mL aliquots of media containing compounds **1** or **22**. Compounds **1** and **22** were dissolved in dimethyl sulfoxide to give stock solutions with concentrations of 1–2 $\mu g\,mL^{-1}$. Stock solutions were diluted with complete normal growth medium to give test solutions with concentrations in the range from 0.2–25 $\mu g\,mL^{-1}$. Culture plates were monitored daily, until control cultures became confluent (typically 4–7 days), at which time all wells were fixed, stained, dried and measured spectrophotometrically. Reported values are the mean of four experimental determinations.

In a separate series of experiments, cultures were grown in microwells to confluence in normal growth medium. Test solutions of compounds **1** and **22** in growth medium were then applied for 24 h. Test and control wells were then washed, and subjected to a Trypan Blue exclusion test. Non-viable cells, identified by blue staining, were counted per random microscopic field. At least eight fields were counted in each experiment.

Acknowledgements

We wish to thank the Foundation for Research, Science and Technology for a New Zealand Research, Science and Technology postdoctoral fellowship for GBE and The University of Auckland Research Committee for financial support for GKS. We would also like to thank Mr Paul Loong and Ms Linda Adams for technical support in performing the in vitro antibacterial assays, and Dr Rod Weston (IRL) for providing an authentic sample of ferruginyl benzoate. Professor Robin Ferrier assisted with the preparation of the manuscript.

References and Notes

1. Neu, H. C. *Science* **1992**, *257*, 1064.
2. Cohen, M. L. *Science* **1992**, *257*, 1050.
3. Muroi, H.; Kubo, I. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1925.
4. Miyamoto, H.; Yamashita, H.; Ueda, H.; Tamaoka, H.; Ohmori, K.; Nakagawa, K. *Bioorg. Med. Chem.* **1995**, *3*, 1699.
5. Bendall, J. G.; Cambie, R. C. *Aust. J. Chem.* **1995**, *48*, 883.
6. Easterfield, T. H. *Trans. New Zealand Inst.* **1911**, *43*, 55.

7. Cambie, R. C.; Mander, L. N. *Tetrahedron* **1962**, *18*, 465.
8. Shimatani, Y., Sakamoto, O., Hayashi, M. Jpn. Kokai Tokkyo Koho, JP 01, 311, 019, 1989. *Chem. Abstr.* **1990**, *112*, 204746.
9. Kubo, I.; Muroi, H.; Himejima, M. *J. Nat. Prod.* **1992**, *55*, 1436.
10. Kubo, I.; Muroi, H.; Kubo, A. *J. Agric. Food Chem.* **1993**, *41*, 2447.
11. Kubo, I.; Muroi, H.; Kubo, A. *J. Nat. Prod.* **1994**, *57*, 9.
12. Muroi, H.; Kubo, I. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1925.
13. Muhammad, I.; Mossa, J. S.; Al-Yahya, M. A.; Ramadan, A. F.; El-Feraly, F. S. *Phytother. Res.* **1995**, *9*, 584.
14. Kubo, I.; Muroi, H.; Kubo, A. *Bioorg. Med. Chem.* **1995**, *3*, 873.
15. Brieskorn, C. H.; Fuchs, A.; Bredenberg, J. B.-S.; McChesney, J. D.; Wenkert, E. *J. Org. Chem.* **1964**, *29*, 2293.
16. Short, W. F., Wang, H. *J. Chem. Soc.* **1951**, 2979.
17. Nishida, T.; Wahlberg, I.; Enzell, C. R. *Org. Magn. Reson.* **1977**, *9*, 203.
18. Compounds labeled with numbers having a, b or c suffixes are synthetic precursors of the products required for the bioassays, the latter being labeled with numbers alone. This manner of numbering allows the compounds to be treated in the experimental section in numerical sequence.
19. Wenkert, E.; Mahajan, J. R.; Nussim, M.; Schenker, F. *Can. J. Chem.* **1966**, *44*, 2575.
20. Lynch, G. P. Ph.D Thesis, University of Otago, 1989.
21. Akita, H.; Oishi, T. *Chem. Pharm. Bull.* **1981**, *29*, 1567.
22. Atwater, N. W. *J. Am. Chem. Soc.* **1960**, *82*, 2847.
23. Kaufman, T. S.; Mischne, M. P.; Gonzalez-Sierra, M.; Ruveda, E. A. *Can. J. Chem.* **1987**, *65*, 2024.
24. Rao, P. N.; Cessac, J. W.; Kim, H. K. *Steroids* **1994**, *59*, 621.
25. Demuynck, M.; De Clercq, P.; Vandewalle, M. *J. Org. Chem.* **1979**, *44*, 4863.
26. Subramanian, L. R., Martinez, A. G., Fernandez, A. H., Alvarez, R. M. *Synthesis* **1984**, 481.
27. Bennett, C. R.; Cambie, R. C. *Tetrahedron* **1966**, *22*, 2845.
28. Burkinshaw, G. F.; Davis, B. R. *Tetrahedron* **1967**, *23*, 4147.
29. Janssen, C. G. M.; Godefroi, E. F. *J. Org. Chem.* **1984**, *49*, 3600.
30. Brandt, C. W., Neubauer, L. G. *J. Chem. Soc.* **1939**, 1031.
31. Bredenberg, J. B.-S. *Acta Chem. Scand.* **1957**, *11*, 932.
32. Fukui, H.; Koshimizu, K.; Egawa, H. *Agric. Biol. Chem.* **1978**, *42*, 1419.
33. Kobayashi, K.; Nishino, C.; Fukushima, M.; Shiobara, Y.; Kodama, M. *Agric. Biol. Chem.* **1988**, *52*, 77.
34. Gonzalez, A. G.; Abad, T.; Jimenez, I. A.; Ravelo, A. G.; Luis, J. G.; Aguiar, Z.; San Andres, L.; Plasencia, M.; Herrera, J. R.; Moujir, L. *Biochem. Syst. Ecol.* **1989**, *17*, 293.
35. Muhammad, I.; Mossa, J. S.; El-Feraly, F. S. *Phytother. Res.* **1992**, *6*, 261.
36. Short, W. F., Stromberg, H. *J. Chem. Soc.* **1937**, 516.
37. Kartha, K. P. R.; Jennings, H. J. J. *Carbohydr. Chem.* **1990**, *9*, 777.
38. Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron* **1987**, *43*, 5919.
39. Jensen, K. J.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2119.
40. Durette, P. L.; Horton, D. *Adv. Carbohydr. Chem. Biochem.* **1971**, *26*, 49.
41. Lien, E. J.; Hansch, C.; Anderson, S. M. *J. Med. Chem.* **1968**, *11*, 430.
42. Katritzky, A. R.; Zhang, Z.; Lan, X.; Lang, H. *J. Org. Chem.* **1994**, *59*, 1900.
43. Haraguchi, H.; Oike, S.; Muroi, H.; Kubo, I. *Planta Med.* **1996**, *62*, 122.
44. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon: Oxford, 1988.
45. Banik, B. K., Chakraborti, A. K., Ghatak, U. R. *J. Chem. Res. (M)* **1986**, 3391.
46. Scott, G. K., Seow, H. F. *Exp. Cell Res.* **1985**, *158*, 41.
47. Scott, G. K.; Tse, C. A. *Biol. Chem. Hoppe-Seyler* **1988**, *369* (Suppl.), 131.