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The Synthesis and Antibacterial Activity of Totarol Derivatives. Part 2: Modifications at C-12 and O-13†

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Abstract—Alterations of the C-12 and C-13 aromatic ring substituents of totarol (**1**) afforded the series of derivatives **2**–**14**, and introduction of substituents at C-12 gave exclusively **2a**–**14a**. The majority of these analogues were tested in vitro against the following organisms: β-lactamase-positive and high level gentamycin-resistant *Enterococcus faecalis,* penicillin-resistant *Streptococcus pneumoniae,* methicillin-resistant *Staphylococcus aureus* (MRSA), and multiresistant *Klebsiella pneumoniae*. The results were evaluated in terms of structure–activity relationship which reveals that: (a) the phenolic moiety at C-13, in general, is essential for antibacterial activity at < 32 μg/mL against Gram-positive species, and (b) derivatization at C-12 has an undesirable effect on the antibacterial activity of this class of compounds, while (c) all compounds tested are ineffective against the Gram-negative *Klebsiella pneumoniae*.

Introduction

The incidence of infection by antibiotic-resistant bacteria has increased to a threatening extent since penicillin-resistant Staphylococci were first encountered in the early 1950s,[1,2] and the resistances to multiple antibiotics of strains of Gram-positive bacteria, e.g., methicillin-resistant *Staphylococcus aureus* (MRSA),[3] *Streptococcus pneumoniae*[4] and *Enterococcus faecalis*[3] are now significant clinical problems. Compounds active against Gram-positive species are thus of significant interest, and totarol (**1**),[5] a phenolic diterpene which is the major constituent of hexane extracts of the heartwood of the New Zealand native tree *Podocarpus totara* G. Benn,[6,7] but which is also available from other sources,[5] is such a compound. It is active against a variety of Gram-positive bacteria,[8–14] notably including MRSA, with which it shows an MIC of 2.7 μM in vitro.[3]

From mode of action studies on two antibiotic diterpenoids structurally related to totarol, pisiferic acid (**15**)[15] and carnosic acid (**16**),[16] it was concluded that their activity is due to their ability to inhibit bacterial peptidoglycan synthesis. Another possible mode of action of lipophilic phenols may involve their ability to uncouple oxidative phosphorylation in mitochondria.[17] Totarol itself inhibits oxygen consumption and respiratory-driven proton translocation in whole cells of a Gram-negative *Pseudomonas aeruginosa* and also NADH oxidation in membrane preparations from this bacterium.[18]

We have been engaged in studies of totarol derivatives with the objectives of identifying the structural features on which the biological activity depends and of enhancing understanding of their mode of action. In Part I of this series[19] we attempted (a) to elucidate the minimum structural requirements for the antibacterial activity of totarol (**1**) by investigating a variety of analogues and homologues carrying modifications at O-13 and C-14, and (b) to enhance the bioavailability of totarol derivatives in vivo by glycosylation at O-13. We now report a comparative investigation of the antibacterial properties of totarol (**1**) and of analogues **2**–**10** and **2a**–**14a** which bear new substituents at C-12 and also, in the former set, O-13.

Results and Discussion

Synthesis of compounds 2–14 and 2a–14a

Since the introduction of electron-withdrawing groups into the aromatic rings of phenols enhances their antiseptic properties,[20] and also since nitration might augment any ability to uncouple bacterial oxidative phosphorylation,[17] the known nitro-compound **2a**[21] was made by the action of nitric acid in acetic acid on totarol (**1**); *O*-methylation of the product gave the known **2**.[22]

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†For Part I see reference 19.

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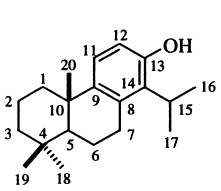

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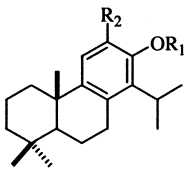
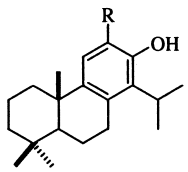
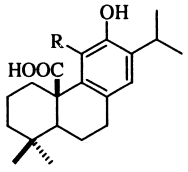


	R_1	R_2
2	CH_3	NO_2
3	CH_3	Br
4	CH_3	NH_2
5	$COCH_3$	$NHCOCH_3$
6	CH_3	OH
7	CH_3	CHO
8	CH_3	COOH
9	CH_3	CH_2OH
10	CH_3	CH_3
11	CH_3	C_2H_5
12	CH_3	$COCH_3$
13	CH_3	$C(CH_3)_2OH$
14	CH_3	$CH(CH_3)_2$



	R
2a	NO_2
3a	Br
4a	NH_2
5a	$NHCOCH_3$
6a	OH
7a	CHO
8a	COOH
9a	CH_2OH
10a	CH_3
11a	C_2H_5
12a	$COCH_3$
13a	$C(CH_3)_2OH$
14a	$CH(CH_3)_2$



	R	
15	H	Pisiferic acid
16	OH	Carnosic acid

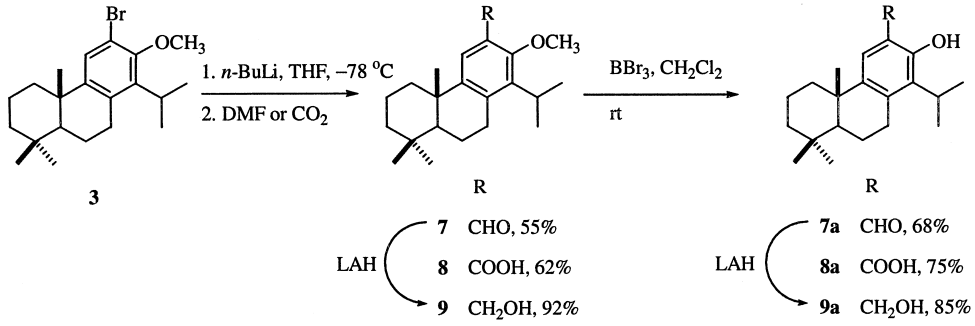
The analogous known 12-bromo-compounds **3a**[23] and **3**[22] were made directly by electrophilic bromination reactions. Reduction of **2** and **2a** led to the known amino-compounds **4**[22] and **4a**,[21] the latter product affording the *N,O*-diacetyl derivative **5** on treatment with acetic anhydride in pyridine, and the specifically *N*-substituted acetanilide **5a** with acetic anhydride in methanol.

Compounds **4a** and **5a** offered the opportunity to investigate the effect of intramolecular hydrogen bonding of the C-13 phenolic group on antibacterial activity, and likewise for this reason catechol **6a**[22] was made from the hydroxyamine **4a** by treatment with sodium periodate in acetic acid. Diazotization of the methoxyamine **4** led to the methoxyphenol **6** (dispermol[24]). It is noteworthy that carnosic acid (**16**), found in rosemary (*Rosmarinus officinalis*, Linn.) leaves[25] and which is structurally similar to catechol **6a**, shows antibiotic activity.[26]

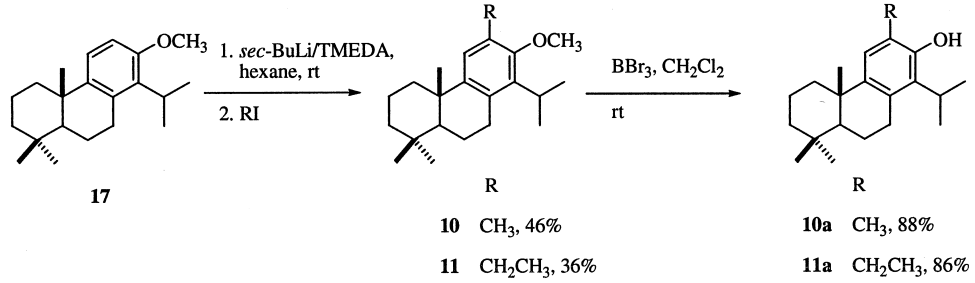
The effects on the antibiotic activity of totarol (**1**) of introducing single carbon groups, in each of the different oxidized states at C-12, were also investigated. In the event that the formyl, carboxy and hydroxymethyl groups by themselves confer activity upon the basic totarol skeleton, the properties of the phenols **7a**–**9a** and their methyl ethers **7**–**9** were compared. Lithiation of compound **3** with *n*-BuLi followed by quenching separately with DMF and solid CO_2 led to the *O*-protected formyl derivative **7** (55%) and carboxy derivative **8** (62%). Compound **7** exhibited resonances in the ^1H and ^{13}C NMR spectra characteristic of an aldehydic group (δ_H 10.18 (1H, s); δ_C 190.6 (d)) on a penta-substituted aromatic ring (δ_H 7.58 (1H, s, H-11); δ_C 124.3 (d, C-11)); the carboxy derivative **8** exhibited resonances in the ^1H and ^{13}C NMR spectra characteristic of a carboxylic acid group (δ_C 169.0 (s)) on a similar ring (δ_H 7.85 (1H, s, H-11); δ_C 119.7 (d, C-11)). Lithium aluminum hydride reduction of the formyl derivative **7** afforded the hydroxymethyl analogue **9** which gave resonances typical of a hydroxymethyl group (δ_H 4.70 (2H, s); δ_C 62.1 (t)) on a pentasubstituted aromatic ring (δ_H 7.14 (1H, s, H-11); δ_C 123.6 (d, C-11)). Demethylation of **7** and **8** was carried out using boron tribromide in dichloromethane[27] to afford **7a** and **8a** in 68% and 75% yield, respectively, and lithium aluminum hydride reduction of hydroxyaldehyde **7a** gave the diol **9a** (85%) (Scheme 1).

Several C-12 alkyl compounds were made to probe the possible relationship between antibacterial activity and lipophilicity, *ortho*-directed metalation[28] being adopted to permit the introduction of a methyl and an ethyl group at this position. Thus 13-methoxytotara-8,11,13-triene (**17**)[29] was treated sequentially with *seco*-BuLi precomplexed with tetra-*N*-methyl(ethylenediamine) and methyl or ethyl iodide at −78 °C in hexane to give the 12-methyl and 12-ethyl derivatives **10** and **11** in 46 and 36% yield, respectively. De-*O*-methylation by use of boron tribromide in dichloromethane afforded phenols **10a** (88%) and **11a** (86%), respectively (Scheme 2).

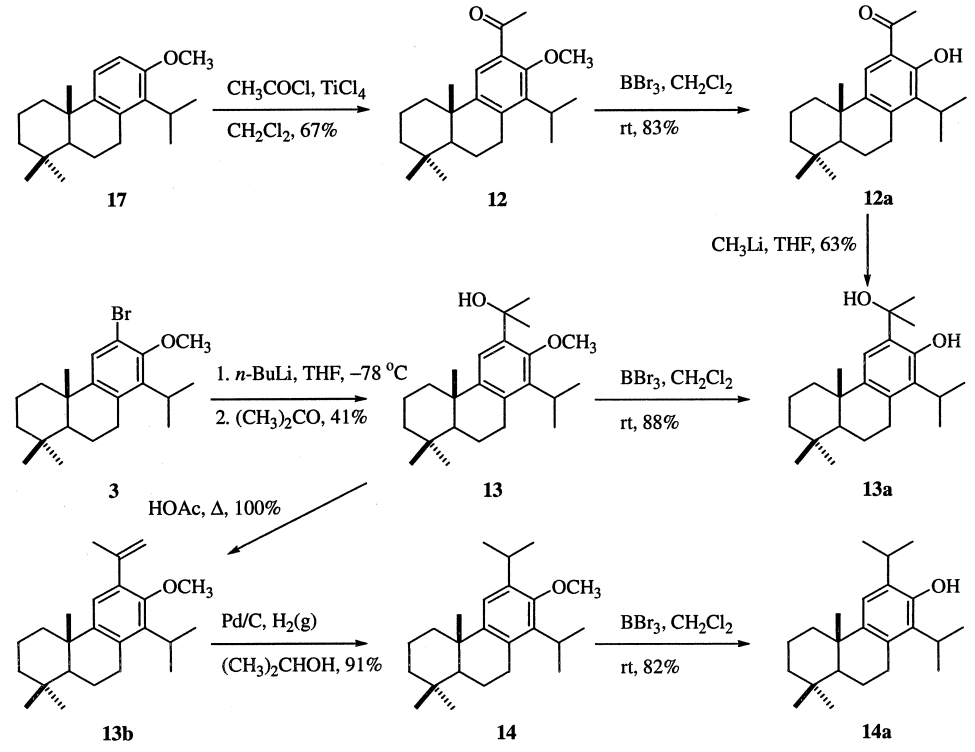
Analogous synthesis of the isopropyl derivatives **13** and **14** was more problematic because a suitable electrophile could not be found for direct introduction of the *C*-alkyl groups. Therefore, an indirect route was adopted via the 12-ketone **12**[30] which was demethylated to give **12a**,[30] and this with methyllithium gave 2-hydroxyprop-2-yl compound **13a** (Scheme 3). Otherwise, **13a** was made from its C-13 methyl ether **13**, which was derived



Scheme 1.



Scheme 2.



Scheme 3.

from **3** by lithiation followed by reaction with acetone. The structure of **13a** was confirmed by its NMR resonances, which were characteristic of a 2-hydroxyprop-2-yl group (δ_H 1.69 (6H, s); δ_C 76.3 (s), 30.3, 30.2 (2q)) bonded to a penta-substituted aromatic ring (δ_H 6.92 (1H, s, H-11); δ_C 118.9 (d, C-11)). Quantitative dehydration of **13** was achieved by heating in acetic acid, and the product **13b** showed resonances in the ^1H and ^{13}C NMR spectra characteristic of a propenyl group (δ_H 5.10 (2H, brs), 2.15 (3H, s); δ_C 145.5 (s), 114.4 (t), 22.8 (q)) attached to a penta-substituted aromatic ring (δ_H 6.98 (1H, s, H-11); δ_C 124.2 (d, C-11)). Hydrogenation over palladium on carbon gave 12-isopropyl-13-methoxytotara-8,11,13-triene (**14**, 91%). Demethylation of

14 using boron tribromide in dichloromethane provided the desired phenol **14a** (82%) (Scheme 3).

Evaluation of in vitro antibacterial activities and relationships to structural factors

Listed in Table 1 are the minimum inhibitory concentrations of compounds **2**–**10** and **2a**–**14a** together with those of the reference compounds totarol (**1**), 2,4-dinitrophenol and carnosic acid (**16**), against the Gram-positive β-lactamase positive and high level gentamycin-resistant *Enterococcus faecalis*, penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), and the Gram-negative multiresistant *Klebsiella pneumoniae*.

As with totarol (**1**) itself, none of the derivatives displayed activity against the Gram-negative organism *Klebsiella pneumoniae*, up to the highest concentration tested (32 μg/mL). Furthermore, none of the analogues retained totarol's potency against all three Gram-positive bacteria. The key conclusions are:

1. the best tolerated C-12 substituents are the hydroxy group (compound **6a**) and formyl, carboxy and hydroxymethyl groups (compounds **7a**–**9a**);
2. an electron-withdrawing nitro-substituent at C-12 (**2a**) strongly diminishes activity, which is consistent with other evidence that the antibacterial properties are not a result of decoupling of oxidative phosphorylation;[17]
3. lipophilic substituents at C-12 (Me, Et, *i*-Pr in **10a**, **11a** and **14a**, respectively) also diminish activity, but they do not eliminate it;
4. *O*-methylation of the phenolic hydroxyl group usually reduced activity appreciably (compounds **2**–**4**, **6**–**10** compared with **2a**–**4a**, **6a**–**10a**, respectively);
5. activity against *Streptococcus pneumoniae* is less vulnerable to substitution than is the activity against the other two Gram-positive strains.

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

Compound	*Enterococcus faecalis*	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
1	2 (7)	2 (7)	2 (7)	> 32
2	> 32	32 (93)	> 32	> 32
3	> 32	> 32	> 32	> 32
4	> 32	32 (102)	> 32	> 32
5	> 32	2 (5)	> 32	> 32
6	> 32	> 32	> 32	> 32
7	> 32	8 (25)	> 32	> 32
8	32 (93)	32 (93)	32 (93)	> 32
9	> 32	> 32	> 32	> 32
10	> 32	> 32	> 32	> 32
2a	> 32	32 (97)	> 32	> 32
3a	> 32	2 (5)	> 32	> 32
4a	> 32	8 (27)	> 32	> 32
5a	> 32	> 32	> 32	> 32
6a	32 (106)	8 (26)	8 (26)	> 32
7a	32 102)	2 (6)	8 (26)	> 32
8a	8 (24)	2 (6)	8 (24)	> 32
9a	8 (25)	2 (6)	> 32	> 32
10a	> 32	2 (7)	32 (107)	> 32
11a	> 32	32 (102)	32 (102)	> 32
12a	> 32	8 (24)	> 32	> 32
13a	8 (23)	2 (6)	32 (23)	> 32
14a	32 (98)	2 (6)	32 (98)	> 32
Carnosic acid (**16**)	8 (24)	8 (24)	32 (96)	> 32
2,4-Dinitrophenol	> 32	> 32	> 32	> 32

[a]Minimum inhibitory concentrations.

In conclusion, it appears that derivatization of the aromatic ring of totarol (**1**) at C-12 has an undesirable effect on its antibacterial activity in vitro. The carboxy derivative **8a**, which retains appreciable activity, may do so because it has greater water solubility and hence better in vivo bioavailability.

Experimental

General methods and syntheses

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer and assignments were made with the assistance of DEPT and COSY experiments. Infrared spectra were obtained (KBr) on a Perkin–Elmer 1600 series FTIR spectrometer. Elemental analyses were conducted with a Carlo Erba EA 1108 elemental analyzer. Low and high resolution (HRMS) mass spectra 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 Merck 60 PF_{254} silica gel-coated aluminum sheets. Purification by flash column chromatography was conducted using Merck Kieselgel S silica gel. Solvents were dried and purified before use according to standard procedures.[31] "Petrol" refers to the fraction of petroleum ether boiling between 60 and 80 °C.

12-Nitrototara-8,11,13-trien-13-ol (2a).[21] A solution of nitric acid (1.5 mL, 23 mmol) in acetic acid (20 mL) was added to a stirred suspension of totarol (**1**, 7.0 g, 24 mmol) in acetic acid (70 mL) at 0 °C. The resulting solution was stirred for 10 min, the reaction was quenched by the addition of ice water (900 mL), and the mixture filtered to yield a yellow solid precipitate. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave **2a** (5.2 g, 64%); mp 78–79 °C, lit.[21] 73–74.5 °C; ^{1}H NMR ($CDCl_3$) δ 10.98 (1H, s, OH), 7.90 (1H, s, H-11), 3.33 (1H, m, H-15), 2.79 (2H, m, H-7), 1.38, 1.35 (2×3H, 2d, J = 6.9 Hz, H-16, H-17), 1.17 (3H, s, H-20), 0.96 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 151.9, 144.7, 143.2, 135.0, 132.1, 118.4 (Ar), 49.0 (C-5), 41.3 (C-3), 39.5 (C-1), 37.8 (C-10), 33.2 (C-4), 33.0 (C-18), 29.2 (C-7), 27.9 (C-15), 25.0 (C-20), 21.6 (C-19), 19.5, 19.5 (C-16, C-17), 19.2 (C-6), 18.8 (C-2).

13-Methoxy-12-nitrototara-8,11,13-triene (2).[22] Methyl iodide (0.94 mL 15 mmol) was added to a stirred suspension of sodium hydride (60% in oil, 0.34 g, 8.5 mmol) and **2a** (2.5 g, 7.6 mmol) in DMF (20 mL) at 0 °C under argon. The suspension 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 with petrol:ethyl acetate (19:1) as the eluent gave **2** (2.4 g, 92%); mp 104–105 °C, lit.[22] mp 104–106 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (1H, s, H-11), 3.76 (3H, s, OCH_3), 3.36 (1H, m, H-15), 2.98 (1H, dd, J = 17.9, 5.8 Hz, H-7β), 2.79 (1H, m, H-7α), 2.24 (1H, br d, J = 12.3 Hz, H-1β), 1.35, 1.32 (2×3H, 2d, J = 6.9 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 150.4, 146.4, 141.7, 140.9, 140.9, 119.9 (Ar), 61.8 (OCH_3), 49.1 (C-5), 41.4 (C-3), 39.3 (C-1), 38.2 (C-10), 33.4 (C-4), 33.1 (C-18), 29.0 (C-7), 27.9 (C-15), 24.9 (C-20), 21.5 (C-19), 21.1, 21.1 (C-16, C-17), 19.2 (C-6), 18.8 (C-2).

12-Bromo-13-methoxytotara-8,11,13-triene (3).[22] A solution of bromine (1.0 g, 6.3 mmol) in acetic acid (10 mL) was added dropwise to a stirred solution of 13-methoxytotara-8,11,13-triene **17**[19] (1.0 g, 3.5 mmol) in diethyl ether (10 mL). After stirring for 3 h the volatiles were removed in vacuo and the residue was recrystallized from methanol to yield **3** as a solid (1.12 g, 84%); mp 130–131 °C, lit.[22] 127.5–129.5 °C; ^{1}H NMR spectral chemical shifts were identical to published data (δ±0.1 ppm).[22]

12-Bromototara-8,11,13-trien-13-ol (3a).[23] Bromine (0.11 mL, 2.0 mmol) was added dropwise to a stirred, ice cooled solution of totarol (**1**, 0.6 g, 2.1 mmol) in CH_2Cl_2 (10 mL). After stirring for 1 h the volatiles were removed in vacuo. Chromatography on silica gel using petrol:ethyl acetate (100:1) as the eluent gave **3a** as an oil (635 mg, 83%). ^{1}H NMR spectral chemical shifts were identical to published data (δ±0.1 ppm).[23]

12-Aminototara-8,11,13-trien-13-ol (4a).[21] Sodium borohydride (50 mg) was added portionwise to a stirred suspension of **2a** (90 mg, 0.27 mmol) and palladium on charcoal (10%, 20 mg) in methanol at room temperature under argon. The mixture was stirred for 30 min, filtered through Celite, and concentrated in vacuo. Chromatography on silica gel using CH_2Cl_2 as the eluent gave **4a** (76 mg, 93%); mp 165–166 °C, lit.[21] mp 165–166 °C; ^{1}H NMR ($CDCl_3$) δ 6.68 (1H, m, H-11), 3.26 (1H, sept, J = 7.1 Hz, H-15), 2.98 (1H, dd, J = 17.9, 5.8 Hz, H-7β), 2.79 (1H, m, H-7α), 1.35, 1.33 (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 1.16 (3H, s, H-20), 0.94 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 144.3, 142.8, 131.4, 130.5, 126.0, 114.3 (Ar), 49.8 (C-5), 41.7 (C-3), 39.7 (C-1), 37.7 (C-10), 33.3 (C-18), 33.3 (C-4), 28.3 (C-7), 27.3 (C-15), 25.2 (C-20), 21.6, 21.6 (C-16, C-17), 20.6 (C-19), 19.6 (C-6), 19.5 (C-2).

12-Amino-13-methoxytotara-8,11,13-triene (4).[22] Sodium borohydride (1.50 g) was added portionwise to a stirred suspension of **2** (1.55 g, 4.5 mmol) and palladium on carbon (10%, 500 mg) in methanol (50 mL) at room temperature under argon. The mixture was stirred for 1 h, filtered through Celite, and concentrated in vacuo. Chromatography on silica gel with CH_2Cl_2 as eluent gave **4** (1.36 g, 96%); mp 137–138 °C, lit.[22] 136–138 °C; ^{1}H NMR spectral chemical shifts were identical to published data (δ±0.1 ppm)[22]; ^{13}C NMR ($CDCl_3$) δ 146.5, 138.0, 137.3, 110.6 (Ar), 59.5 (OCH_3), 49.5 (C-5), 41.5 (C-3), 39.4 (C-1), 37.8 (C-10), 33.2 (C-4), 33.1 (C-18), 27.9 (C-7), 27.3 (C-15), 24.8 (C-20), 21.7, 21.7 (C-16, C-17), 21.5 (C-19), 19.4 (C-6), 19.4 (C-2).

12-Acetamido-13-acetoxytotara-8,11,13-triene (5). Acetic anhydride (2.0 mL) was added to a stirred solution of **4a** (150 mg, 0.5 mmol) in pyridine (2.0 mL) at 0 °C. The resulting solution was allowed to warm to room temperature, stirred for 14 h, diluted with water (100 mL), extracted with ethyl acetate (3×50 mL) and the combined organic extracts were washed with brine (3×25 mL), dried ($MgSO_4$) and concentrated in vacuo. Silica gel chromatography using petrol:ethyl acetate (1.5:1) as the eluent gave **5** (136 mg, 71%); mp 214–216 °C; ^{1}H NMR ($CDCl_3$) δ 6.84 (1H, s, H-11), 3.28 (1H, sept, J = 7.0 Hz, H-15), 2.94–2.66 (2H, m, H-7), 2.34 (3H, s, $NCOCH_3$), 2.09 (3H, s, $COCH_3$), 1.22, 1.22 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.20 (3H, s, H-20), 0.94 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 169.6, 168.4 (*C*OCH_3), 148.6, 136.9, 132.0, 127.2, 123.3, 121.2 (Ar), 49.1 (C-5), 41.3 (C-3), 39.2 (C-1), 38.1 (C-10), 33.2 (C-4), 33.0 (C-18), 28.3 (C-7), 27.2 (C-15), 24.8 (C-20), 23.8 ($NCOCH_3$), 21.4 (C-19), 21.1 ($OCOCH_3$), 20.8, 20.7 (C-16, C-17), 19.2 (C-6), 19.0 (C-2); m/z 385 (M^+, 5%), 325 (82), 310 (100), 301 (58), 286 (12), 242 (24), 228 (45), 214 (59); HRMS m/z calcd for $C_{24}H_{35}NO_3$ (M^+) 385.2616, found 385.2615.

12-Acetamidototara-8,11,13-trien-13-ol (5a). Acetic anhydride (1.0 mL) was added dropwise to a stirred solution of **4a** (114 mg, 0.38 mmol) in methanol (10 mL) at room temperature under argon. The mixture was stirred for 14 h, the volatiles were removed under reduced pressure and the resulting residue was redissolved in ethyl acetate, washed with brine (3×25 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave **5a** as an oil; ν_{max} 3501 (OH), 1626 (CO), 1545 (NCO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.17 (1H, s, OH), 7.57 (1H, s, NH), 6.69 (1H, m, H-11), 3.27 (1H, m, H-15), 2.92, (1H, dd, J = 17.1, 6.1 Hz, H-7β), 2.73 (1H, ddd, J = 17.1, 11.3 7.8 Hz, H-7), 2.20 (3H, s, $NCOCH_3$), 1.37, 1.36 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.11 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 170.4 (NCO), 146.1, 142.9, 135.7, 132.1, 124.3, 116.4 (Ar), 49.5 (C-5), 41.5 (C-3), 39.7 (C-1), 37.6 (C-10), 33.3 (C-4), 33.2 (C-18), 28.5 (C-7), 28.0 (C-15), 25.2 (C-20), 23.5 ($NCOCH_3$), 21.6 (C-19), 20.2, 20.2 (C-16, C-17), 19.4 (C-6), 19.3 (C-2); m/z 343 (M^+, 84%), 325 (56), 310 (68), 301 (100), 286 (31), 240 (53), 228 (48), 214 (75), 190 (26), 69 (22), 55 (21), 41 (32); HRMS m/z calcd for $C_{22}H_{33}NO_2$ (M^+) 343.2511, found 343.2514.

13-Methoxytotara-8,11,13-trien-12-ol (6).[24] To a stirred solution of **4** (100 mg, 0.3 mmol) in methanol excess isoamyl nitrite (0.3 mL) and HCl (0.1 mL, concd) were added. The reaction mixture was stirred at room temperature overnight, diluted with ethyl acetate (100 mL) and washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave **6** (79 mg, 79%); mp 163–165 °C, lit.[24] 166.5–167.5 °C. ^{1}H NMR

spectral chemical shifts were identical to published data (δ±0.1 ppm).[24]

Totara-8,11,13-triene-12,13-diol (6a).[32] A solution of **4a** (320 mg, 1.06 mmol) in acetic acid (90 mL) was added over a period of 3 min to a stirred solution of sodium periodate (3.0 g) in dilute HCl (0.1 M, 210 mL) at room temperature. The reaction mixture was stirred for 5 min, extracted with chloroform (3×50 mL) and the combined organic extracts were washed with brine (3×25 mL) and shaken with potassium iodide (0.9 g) in acetic acid (2.0 mL) for 2 min. The resulting solution was washed with aq $NaHSO_4$ (2×50 mL), brine (2×50 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave **6a** as an oil (260 mg, 81%); ^{1}H NMR spectral chemical shifts were identical to published data (δ±0.1 ppm);[32] ^{13}C NMR ($CDCl_3$) δ 147.2, 146.8, 144.2, 138.2, 125.7, 110.0 (Ar), 49.6 (C-5), 41.6 (C-3), 39.5 (C-1), 38.1 (C-10), 33.4 (C-4), 33.2 (C-18), 28.0 (C-7), 27.5 (C-15), 24.8 (C-20), 21.7 (C-19), 21.6, 21.6 (C-16, C-17), 19.5 (C-6), 19.5 (C-2).

12-Formyl-13-methoxytotara-8,11,13-triene (7). A stirred solution of bromide **3** (216 mg, 0.78 mmol) in THF (10 mL) was cooled to −78 °C and treated dropwise with a solution of *n*-BuLi (0.68 mL, 1.3 M in hexanes, 0.88 mmol) and the resulting mixture stirred for an additional 10 min at −78 °C. The reaction was then quenched with DMF (1 mL) and allowed to warm to room temperature and stirred for an additional 30 min at this temperature. The mixture was diluted with aqueous ammonium chloride (10% w/w, 100 mL) and extracted with ethyl acetate (3×50 mL) and the combined organic layers were washed with brine (3×50 mL), dried ($MgSO_4$) and concentrated in vacuo to give an oily residue. Chromatography on silica gel using petrol: ethyl acetate (19:1) as the eluent gave **7** as an oil (142 mg, 55%); ν_{max} 1697 (CO), 1592 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.18 (1H, s, CHO), 7.58 (1H, m, H-11), 3.80 (3H, s, OCH_3), 3.30 (1H, br s, H-15), 2.93 (1H, dd, J=17.9, 5.7 Hz, H-7β), 2.76 (1H, ddd, J=17.9, 10.5, 8.1 Hz, H-7), 1.32, 1.31 (2×3H, 2d, J=7.0 Hz, H-16, H-17), 1.11 (3H, s, H-20), 0.88 (3H, s, H-18), 0.86 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 190.6 (CHO), 147.1, 139.3, 127.2, 124.3 (Ar), 65.5 (OCH_3), 49.4 (C-5), 41.6 (C-3), 39.6 (C-1), 38.3 (C-10), 33.5 (C-4), 33.3 (C-18), 29.4 (C-7), 27.4 (C-15), 25.0 (C-20), 21.7 (C-19), 21.6, 21.6 (C-16, C-17), 19.4 (C-6), 19.2 (C-2); HRMS m/z calcd for $C_{22}H_{32}O_2$ (M^+) 328.2402, found 328.2397.

12-Formyltotara-8,11,13-trien-13-ol (7a). Boron tribromide (1.0 M, 5 mL)[27] was added dropwise to a stirred solution of **7** (500 mg, 1.5 mmol) in CH_2Cl_2 (20 mL) at room temperature under argon. The mixture was stirred for 30 min, diluted with CH_2Cl_2 (100 mL), washed with aq $Na_2S_2O_3$ (10% w/w, 3×25 mL), aq $NaHCO_3$ (3×25 mL), brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol: ethyl acetate (4:1) as the eluent gave **7a** (322 mg, 68%); mp 119 °C; ν_{max} 3684 (OH), 1688 (CO), 1592 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.15 (1H, s, OH), 9.78 (1H, s, CHO), 7.30 (1H, s, H-11), 3.29 (1H, sept, J=6.9 Hz, H-15), 3.01 (1H, dd, J=18.3, 5.9 Hz, H-7β), 2.81 (1H, ddd, J=18.3, 10.9, 8.1 Hz, H-7), 1.38, 1.36 (2 3H, 2d, J=6.9 Hz, H-16, H-17), 1.20 (3H, s, H-20), 0.98 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 196.7 (CHO), 158.3, 143.8, 142.8, 133.0, 128.0, 119.2 (Ar), 49.3 (C-5), 41.6 (C-3), 39.8 (C-1), 37.7 (C-10), 33.4 (C-4), 33.2 (C-18), 29.5 (C-7), 27.5 (C-15), 25.3 (C-20), 21.7 (C-19), 19.8, 19.8 (C-16, C-17), 19.4 (C-6), 19.1 (C-2); m/z 314 (M^+, 51%), 299 (100), 256 (45), 229 (50), 217 (35), 203 (65), 169 (36), 128 (33), 64 (87), 41 (24); anal. calcd for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6; found: C, 80.3; H, 9.8.

12-Carboxy-13-methoxytotara-8,11,13-triene (8). A stirred solution of compound **3** (216 mg, 0.78 mmol) in THF (10 mL) was cooled to −78 °C and treated dropwise with a solution of *n*-BuLi (0.68 mL, 1.3 M in hexanes, 0.88 mmol) and the resulting mixture stirred for an additional 10 min at −78 °C. The reaction was then quenched with solid CO_2 (1 g) and allowed to warm to room temperature and stirred for an additional 30 min at this temperature. The mixture was diluted with aq ammonium chloride (10% w/w, 100 mL), extracted with ethyl acetate (3×50 mL) and the combined organic layers were washed with brine (3×50 mL), dried ($MgSO_4$) and concentrated in vacuo to give an oily residue. Chromatography on silica gel using petrol:ethyl acetate (1:1) as the eluent gave **8** (165 mg, 62%); mp 179 °C; ν_{max} 3198 (OH), 1728 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, s, H-11), 3.84 (3H, s, OCH_3), 3.43 (1H, sept, J=7.1 Hz, H-15), 3.01 (1H, dd, J=17.8, 5.3 Hz, H-7β), 2.84 (1H, ddd, J=17.8, 10.5, 7.9 Hz, H-7), 1.36, 1.35 (2×3H, 2d, J=7.1 Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.96 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 156.0, 147.0, 141.6, 138.9, 127.1, 119.7 (Ar), 63.4 (OCH_3), 49.2 (C-5), 41.3 (C-3), 39.2 (C-1), 38.0 (C-10), 33.2 (C-4), 33.0 (C-18), 28.8 (C-7), 27.0 (C-15), 24.6 (C-20), 21.4 (C-19), 21.3, 21.2 (C-16, C-17), 19.1 (C-6), 18.9 (C-2); m/z 344 (M^+, 72%), 329 (100), 311 (26), 269 (25), 259 (64), 247 (47), 233 (82), 69 (27), 41 (29); anal. calcd for $C_{22}H_{32}O_3$: C, 76.7; H, 9.4; found: C, 76.7; H, 9.4.

12-Carboxytotara-8,11,13-trien-13-ol (8a). Boron tribromide (1.0 M in CH_2Cl_2, 1 mL) was added dropwise to a stirred solution of **8** (60 mg, 0.17 mmol) in CH_2Cl_2 (5 mL) at room temperature under an inert atmosphere. The mixture was stirred for 30 min, diluted with CH_2Cl_2 (50 mL), washed with aq $Na_2S_2O_3$ (10%, 3×25 mL), aq $NaHCO_3$ (3×25 mL) and brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (2.5:1) as the eluent gave **8a** (42 mg, 75%) as an oil; ν_{max} 3108 (OH), 1647 (CO), 1600 (C=C), 1446 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.86 (1H, br s, COOH), 7.68 (1H, s, H-11), 3.24 (1H, m, H-15), 2.85 (2H, m, H-7αβ), 1.30 (2×3H, t, J=7.2 Hz, H-16, H-17), 1.13 (3H, s, H-20), 0.93 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 171.7 (COOH), 158.9, 142.1, 141.6, 132.9, 124.5 (Ar), 49.4 (C-5), 41.6 (C-3), 39.6 (C-1), 37.6 (C-10), 33.3 (C-18), 33.2 (C-4), 29.2 (C-7), 27.0 (C-15), 25.1 (C-20), 21.6, 21.1 (C-16, C-17), 19.8 (C-19), 19.4 (C-6), 19.2 (C-2); m/z 330 (M^+, 42%), 315 (100), 312 (40), 297 (22), 245 (21), 219 (35), 201 (31), 69 (64), 41 (88); HRMS m/z calcd for $C_{21}H_{30}O_3$ (M^+) 330.2195, found 330.2193.

12-Hydroxymethyl-13-methoxytotara-8,11,13-triene (9). Lithium aluminum hydride (100 mg) was added portionwise to a stirred solution of **7** (200 mg, 0.6 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.1 mL), aq NaOH (15%, 0.1 mL), and water (0.3 mL), filtered and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (4:1) as the eluent gave **9** as a solid (93 mg, 40%); mp 101 °C; ν_{max} 3408 (OH), 1600 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.14 (1H, m, H-11), 4.70 (2H, s, CH_2OH), 3.77 (3H, s, OCH_3), 3.40 (1H, br s, H-15), 2.96 (1H, dd, $J=17.2$ Hz, 5.4 Hz, H-7β), 2.78, (1H, ddd, $J=17.2$, 10.6, 7.5 Hz, H-7), 1.33, 1.32 (2×3H, 2d, $J=7.2$ Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.94 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 154.9, 146.6, 138.3, 134.6, 131.2, 123.6 (Ar), 62.1 (OCH_3), 62.1 (CH_2OH), 49.6 (C-5), 41.6 (C-3), 39.6 (C-1), 38.1 (C-10), 33.4 (C-4), 33.2 (C-18), 28.6 (C-7), 27.2 (C-15), 25.0 (C-20), 21.7 (C-19), 21.6, 21.6 (C-16, C-17), 19.5 (C-6), 19.4 (C-2); *m/z* 330 (M^+, 100%), 315 (84), 279 (15), 219 (71), 167 (24), 149 (70), 84 (20), 69 (31); HRMS *m/z* calcd for $C_{22}H_{34}O_2$ (M^+) 330.2559, found 330.2553.

12-(Hydroxymethyl)totara-8,11,13-trien-13-ol (9a). Lithium aluminum hydride (100 mg) was added potionwise to a stirred solution of **7a** (200 mg, 0.64 mmol) in THF (10 mL) under argon at room temperature. The mixture was stirred for 2 h and the reaction was quenched with water (0.1 mL), aq NaOH (15%, 0.1 mL), and water (0.3 mL). After filtration, the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave **9a** (170 mg, 85%); mp 101 °C; ν_{max} 3383 (OH), 1640 (C=C), 1592 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.19 (1H, s, H-11), 4.78 (2H, d, $J=4.6$ Hz, CH_2OH), 3.30 (1H, sept, $J=7.0$ Hz, H-15), 2.95 (1H, dd, $J=17.1$, 6.4 Hz, H-7β), 2.76, (1H, ddd, $J=17.1$, 11.2, 7.8 Hz, H-7), 1.39, 1.37 (2 3H, 2d, $J=7.0$ Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.97 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.8, 141.8, 133.9, 132.1, 122.4, 121.6 (Ar), 65.4 (CH_2OH), 49.5 (C-5), 41.4 (C-3), 39.5 (C-1), 37.4 (C-10), 33.1 (C-4), 33.1 (C-18), 28.5 (C-7), 27.3 (C-15), 25.0 (C-20), 21.4 (C-19), 20.1, 20.1 (C-16, C-17), 19.3 (C-6), 19.2 (C-2); *m/z* 316 (M^+, 80%), 298 (100), 283 (51), 255 (65), 229 (40), 215 (44), 202 (51), 187 (38), 69 (32), anal. calcd for $C_{21}H_{32}O_2$: C, 79.6; H, 10.2; found: C, 79.2; H, 10.2.

13-Methoxy-12-methyltotara-8,11,13-triene (10). *sec*-Butyllithium (6.5 mL, 1.3 M in hexanes, 8.5 mmol) was added dropwise to a stirred solution of $Me_2NCH_2CH_2NMe_2$ (1.3 mL, 8.6 mmol) in hexane (5 mL) at room temperature under argon. The resulting solution was stirred for 30 min and added via a cannula to a solution of **17**[19] (265 mg, 0.86 mmol) in hexane (5 mL) and this was stirred for 14 h. The reaction was quenched by the addition of methyl iodide (1.0 mL), and the solution was diluted with aqueous ammonium chloride (10% w/w, 50 mL), extracted with ethyl acetate (3×50 mL) and the combined organic extracts were washed with brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (50:1) as the eluent gave **10** (127 mg, 46%); mp 105 C; υ_{max} 1637 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.94 (1H, m, H-11), 3.71 (3H, s, OCH_3), 3.35 (1H, m, H-15), 2.97-2.69 (2H, m, H-7), 2.23 (3H, s, CH_3), 1.32, 1.31 (2×3H, 2d, $J=7.1$ Hz, H-16, H-17), 1.18 (3H, s, H-20), 0.94 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 145.8, 137.7, 131.8, 127.9, 125.4 (Ar), 60.6 (OCH_3), 49.6 (C-5), 41.5 (C-3), 39.4 (C-1), 37.8 (C-10), 33.4 (C-4), 33.2 (C-18), 28.4 (C-7), 27.3 (C-15), 24.9 (C-20), 21.7, 21.7 (C-16, C-17), 21.6 (C-19), 19.4 (C-6), 19.4 (C-2), 16.9 (CH_3); *m/z* 314 (M^+, 100%), 300 (99), 229 (35), 203 (81), 187 (14), 91 (10), 69 (24). Anal. calcd for $C_{22}H_{34}O$: C, 84.0; H, 10.9; found: C, 83.8; H, 11.0.

12-Methyltotara-8,11,13-trien-13-ol (10a). Boron tribromide (1.0 M in CH_2Cl_2, 2 mL) was added dropwise to a stirred solution of **10** (51 mg, 0.16 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 aq $Na_2S_2O_3$ (10% w/w, 3×25 mL), aq $NaHCO_3$ (3×25 mL) and brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19.1) as the eluent gave **10a** (42 mg, 88%); mp 115 C; ν_{max} 3500 (OH), 1648 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 (1H, m, H-11), 4.45 (1H, s, OH), 3.35 (1H, m, H-15), 2.90, (1H, dd, $J=16.8$, 6.5 Hz, H-7β), 2.72, (1H, ddd, $J=16.8$, 11.2, 7.8 Hz, H-7), 2.22 (3H, s, CH_3), 1.35, 1.33 (2×3H, 2d, $J=7.1$ 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 150.5, 142.6, 131.5, 130.5, 124.4, 120.7 (Ar), 49.6 (C-5), 41.5 (C-3), 39.6 (C-1), 37.5 (C-10), 33.2 (C-4), 33.2 (C-18), 28.4 (C-7), 27.0 (C-15), 25.1 (C-20), 21.5 (C-19), 20.4, 20.4 (C-16, C-17), 19.4 (C-6), 19.4 (C-2), 15.8 (CH_3); *m/z* 300 (M^+, 63%), 285 (100), 215 (31), 189 (51), 69 (18); HRMS *m/z* calcd for $C_{21}H_{32}O$ (M^+) 300.2453, found 300.2451.

12-Ethyl-13-methoxytotara-8,11,13-triene (11). *s*-Butyllithium (6.5 mL, 1.3 M in hexanes, 8.5 mmol) was added dropwise to a stirred solution of $Me_2NCH_2CH_2NMe_2$ (1.3 mL, 8.6 mmol) in hexane (5 mL) at room temperature under argon. The resulting solution was stirred for 30 min and added via a cannula to a solution of **17** (265 mg, 0.86 mmol) in hexane (5 mL) and this was stirred for 14 h. The reaction was quenched by the addition of ethyl iodide (1.0 mL) and the solution diluted with aqueous ammonium chloride (10% w/w, 50 mL), extracted with ethyl acetate (3×50 mL) and the combined organic extracts washed with brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (50:1) as the eluent gave **11** (105 mg, 36%); mp 91 C; ν_{max} 1638 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.00 (1H, m, H-11), 3.72 (3H, s, OCH_3), 3.39 (1H, br s, H-15), 2.94 (1H, dd, $J=16.7$, 5.9 Hz, H-7β), 2.86 (1H, m, H-7α), 2.63 (2H, q, $J=7.6$ Hz, CH_2CH_3), 1.33, 1.31 (2×3H, 2d, $J=7.2$ Hz, H-16, H-17), 1.23 (3H, t, $J=7.6$ Hz, CH_2CH_3) 1.19 (3H, s, H-20), 0.94 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 146.0, 137.7, 133.9, 131.0, 123.5 (Ar), 61.5 (OCH_3), 49.6 (C-5), 41.5 (C-3), 39.5 (C-1), 38.0 (C-10), 33.4 (C-4), 33.2 (C-18), 28.4 (C-7), 27.2 (C-15), 24.9 (C-20), 22.9 (CH_2CH_3), 21.7, 21.7 (C-16, C-17), 21.6 (C-19), 19.4 (C-6), 19.4 (C-2), 14.8 (CH_2CH_3); *m/z* 328

(M^+, 100%), 313 (95), 271 (14), 243 (30), 217 (62), 69 (17); HRMS *m/z* calcd for $C_{23}H_{36}O$ (M^+) 328.2766, found 328.2765.

12-Ethyltotara-8,11,13-trien-13-ol (11a). Boron tribromide (1.0 M in CH_2Cl_2, 1 mL) was added dropwise to a stirred solution of **11** (28 mg, 0.09 mmol) in CH_2Cl_2 (5 mL) at room temperature under argon. The mixture was stirred for 30 min, diluted with CH_2Cl_2 (50 mL), washed with aq $Na_2S_2O_3$ (10% w/w, 3×25 mL), aq $NaHCO_3$ (3×25 mL), and brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave **11a** as an oil (23 mg, 86%); ν_{max} 3439 (OH), 1639 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.93 (1H, m, H-11), 4.54 (1H, s, OH), 3.32 (1H, br s, H-15), 2.91 (1H, dd, *J* = 16.8, 6.6 Hz, H-7β), 2.72 (1H, m, H-7α), 2.55 (2H, q, *J* = 7.6 Hz, CH_2CH$_3$), 1.35, 1.35 (2×3H, 2d, *J* = 7.0 Hz, H-16, H-17), 1.24 (3H, t, *J* = 7.6 Hz, CH$_2$CH_3), 1.19 (3H, s, H-20), 0.94 (3H, s, H-18), 0.91 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 150.3, 142.9, 131.0, 130.7, 127.1, 122.8 (Ar), 49.9 (C-5), 41.8 (C-3), 39.8 (C-1), 37.9 (C-10), 33.4 (C-4), 33.4 (C-18), 28.8 (C-7), 27.3 (C-15), 25.3 (C-20), 23.2 (*C*H$_2$CH$_3$), 21.7 (C-19), 20.7, 20.7 (C-16, C-17), 19.7 (C-6), 19.6 (C-2), 14.2 (CH$_2$*C*H$_3$); *m/z* 314 (M^+, 26%), 299 (28), 286 (42), 217 (41), 191 (100), 177 (40), 163 (33), 109 (44), 81 (51), 69 (60). Anal. calcd for $C_{22}H_{34}O$: C, 84.0; H, 10.9; found: C, 83.9; H 11.0.

12-Acetyl-13-methoxytotara-8,11,13-triene (12).[30] Titanium tetrachloride (7.5 mL, 1.0 M in CH_2Cl_2) was added dropwise to an ice-cooled, stirred solution of compound **17** (1.0 g, 3.4 mmol) and acetyl chloride (0.54 mL) in CH_2Cl_2 (20 mL) and the resulting mixture was stirred for 30 min and the reaction quenched by the addition of aqueous ammonium chloride (10% w/w, 100 mL). The solution was further diluted with CH_2Cl_2 (50 mL), washed with aq $NaHCO_3$ (3×20 mL), brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified on silica gel to give **12** (780 mg, 67%); mp 154–155 °C, lit.[30] mp 159 °C. ^1H and ^{13}C NMR spectral chemical shifts were identical to published data (δ±0.1 ppm).[30]

12-Acetyltotara-8,11,13-trien-13-ol (12a).[30] Boron tribromide (1.0 M in CH_2Cl_2, 1 mL) was added dropwise to a stirred solution of **12** (50 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) at room temperature under argon. The solution was stirred for 30 min, diluted with CH_2Cl_2 (50 mL), washed with aq $Na_2S_2O_3$ (10%, 3×25 mL), aq $NaHCO_3$ (3×25 mL), and brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (100:1) as the eluent gave **12a** (41 mg, 83%), mp 143–145 °C, lit.[30] 144–146 °C. ^1H and ^{13}C NMR spectral chemical shifts were identical to published data (δ±0.1 ppm).[30]

12-(2-Hydroxyprop-2-yl)-13-methoxytotara-8,11,13-triene (13).[30] A stirred solution of bromo-compound **3** (500 mg, 1.3 mmol) in THF (20 mL) was cooled to −78 °C and treated dropwise with a solution of *n*-butyllithium (1.3 mL, 1.3 M in hexanes, 1.6 mmol) and the resulting mixture stirred for an additional 10 min at −78 °C. The reaction mixture was then quenched with acetone (1.0 mL) and allowed to warm to room temperature and stirred for an additional 30 min. The mixture was diluted with aqueous ammonium chloride (10% w/w, 100 mL), extracted with ethyl acetate (3×50 mL) and the combined organic layers were washed with brine (3×50 mL), dried ($MgSO_4$) and concentrated in vacuo to give an oily residue. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave **13** (190 mg, 41%); mp 149–150 °C; ν_{max} 3439 (OH), 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.10 (1H, s, H-11), 4.96 (1H, s, OH), 3.84 (3H, s, OCH_3), 3.61 (1H, sept, *J* = 7.2 Hz, H-15), 3.04 (1H, dd, *J* = 17.0, 6.4 Hz, H-7β), 2.86, (1H, ddd, 17.0, 10.4, 7.6 Hz, H-7), 1.41 (6H, s, $(CH_3)_2$), 1.40, 1.38 (2 3H, 2d, *J* = 7.0 Hz, H-16, H-17), 1.19 (3H, s, H-20), 0.96 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 153.9, 146.6, 138.3, 137.3, 135.1, 120.9 (Ar), 73.5 (COH), 63.4 (OCH_3), 49.7 (C-5), 41.4 (C-3), 39.4 (C-1), 38.4 (C-10), 33.4 (C-4), 33.2 (C-18), 32.2, 31.9 ((*C*H$_3$)$_2$C), 28.0 (C-7), 26.3 (C-15), 24.8 (C-20), 21.6, 21.5 (C-16,C-17), 21.3 (C-19), 19.4 (C-6), 19.3 (C-2); *m/z* 358 (M^+, 1%), 340 (63), 325 (100), 255 (26), 229 (56), 83 (88), 63 (30). Anal. calcd for $C_{24}H_{38}O_2$: C, 80.4; H, 10.7; found: C, 80.7; H, 10.9.

12-(2-Hydroxyprop-2-yl)totara-8,11,13-trien-13-ol (13a). Methyllithium (1.2 M in diethyl ether, 1.5 mL) was added dropwise to a stirred solution of ketone **12a** (120 mg, 0.37 mmol) in THF (5 mL) at room temperature. After 1 h the reaction mixture was diluted with aqueous ammonium chloride (50 mL, 10%), extracted with ethyl acetate (3×20 mL) and the combined organic layers were washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo to yield an oily residue. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave **13a** as an oil (80 mg, 63%); ν_{max} 3334 (OH), 1633 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.75 (1H, s, OH), 6.92 (1H, s, H-11), 3.29 (1H, m, H-15), 2.95, (1H, dd, *J* = 17.0, 5.9 Hz, H-7β), 2.76 (1H, ddd, *J* = 17.0, 11.3, 7.8 Hz, H-7), 1.69 (6H, s, $(CH_3)_2$), 1.39, 1.38 (2×3H, 2d, *J* = 7.1 Hz, H-16, H-17), 1.21 (3H, s, H-20), 0.97 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.0, 141.1, 133.0, 132.5, 128.5, 118.9 (Ar), 76.3 (COH), 49.7 (C-5), 41.6 (C-3), 39.7 (C-1), 37.7 (C-10), 33.3 (C-4), 33.2 (C-18), 30.3, 30.2 ((*C*H$_3$)$_2$C), 28.6 (C-7), 27.7 (C-15), 25.3 (C-20), 21.6 (C-19), 20.2, 20.2 (C-16, C-17), 19.5 (C-6), 19.4 (C-2); *m/z* 344 (M^+, 2%), 326 (57), 241 (35), 215 (34), 84 (17), 69 (21), 55 (13), 49 (22); HRMS *m/z* calcd for $C_{23}H_{36}O_2$ (M^+) 344.2715, found 344.2718.

13-Methoxy-12-(prop-2-enyl)totara-8,11,13-triene (13b). A solution of **13** (150 mg, 0.4 mmol) in acetic acid (10 mL) was heated under reflux for 5 min and then concentrated under reduced pressure to give **13b** (136 mg, 100%); mp 148 °C; ν_{max} 1633 (C=C), 1233, 1018 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.98 (1H, s, H-11), 5.10 (2H, br s, C=CH$_2$), 3.70 (3H, s, OCH_3), 3.33 (1H, br s, H-15), 2.95, 2.78 (1H, dd, *J* = 16.9, 6.2 Hz, H-7β), 2.78 (1H, ddd, *J* = 16.9, 11.0, 7.9 Hz, H-7), 2.15 (3H, s, CH$_3$C=C), 1.35, 1.34 (2×3H, 2d, *J* = 7.1 Hz, H-16, H-17), 1.23 (3H, s, H-20), 0.97 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 145.7 (Ar), 145.5 (C=CH$_2$), 137.9,

137.3, 134.3, 124.2 (Ar), 114.4 (CH_2=C), 60.3 (OCH_3), 49.5 (C-5), 41.6 (C-3), 39.5 (C-1), 37.9 (C-10), 33.3 (C-4), 33.2 (C-18), 28.7 (C-7), 27.6 (C-15), 25.0 (C-20), 22.8 (CH_3C=C), 21.6, 21.6 (C-16, C-17), 21.6 (C-19), 19.5 (C-6), 19.4 (C-2); *m/z* 340 (M^+, 77%), 325 (100), 283 (18), 255 (33), 229 (73), 69 (15). Anal. calcd for $C_{24}H_{36}O$: C, 84.7; H, 10.7; found: C, 84.7; H, 10.9.

12-Isopropyl-13-methoxytotara-8,11,13-triene (14). A mixture of **13b** (100 mg, 0.29 mmol), Pd/C (10%, 50 mg), isopropanol (5 mL) and hexane (5 mL) was stirred at room temperature under an atmosphere of hydrogen for 3 h, after which the solids were removed by filtration through Celite and the volatiles were removed in vacuo. The residue was chromatographed on silica gel using petrol:ethyl acetate (100:1) and the eluent gave **14** (92 mg, 91%); mp 145 °C; ^{1}H NMR ($CDCl_3$) δ 7.08 (1H, s, H-11), 3.74 (3H, s, OCH_3), 3.46 (1H, br s, H-15), 3.30 (1H, sept, *J* = 6.9 Hz, $(CH_3)_2CH$), 2.96 (1H, dd, *J* = 16.1, 5.7 Hz, H-7β), 2.80 (1H, ddd, *J* = 16.1, 10.9, 7.8 Hz, H-7), 1.37, 1.36 (2×3H, 2d, *J* = 6.9 Hz, H-16, H-17), 1.25, 1.23 (2 3H, 2d, *J* = 6.9 Hz, $CH(CH_3)_2$), 1.22 (3H, s, H-20), 0.97 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 154.0, 138.9, 137.6, 137.6, 120.8 (Ar), 62.4 (OCH_3), 49.7 (C-5), 41.6 (C-3), 39.5 (C-1), 38.3 (C-10), 33.4 (C-4), 33.2 (C-18), 28.4 (C-7), 27.3 (C-15), 26.4 ($CH(CH_3)_2$), 25.0 (C-20), 21.8 (C-19), 21.6, 21.6 (C-16, C-17), 19.5 (C-6), 19.5 (C-2); *m/z* 342 (M^+, 64%), 327 (100), 257 (39), 231 (82), 69 (18); anal. calcd for $C_{24}H_{38}O$: C, 84.2; H, 11.2; found: C, 84.0; H, 11.4.

12-Isopropyltotara-8,11,13-trien-13-ol (14a). Boron tribromide (1.0 M in CH_2Cl_2, 1 mL) was added dropwise to a stirred solution of **14** (60 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) at room temperature under argon. The mixture was stirred for 30 min, diluted with CH_2Cl_2 (100 mL), washed with aq $Na_2S_2O_3$ (10% w/w, 3×25 mL), aq $NaHCO_3$ (3×25 mL), and brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo to give a solid. Recrystallization from ethyl acetate gave **14a** (47 mg, 82%), mp 114 °C; ν_{max} 3431 (OH), 1630 (C=C), 1215 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.02 (1H, s, H-11), 4.65 (1H, s, OH), 3.36 (1H, sept, *J* = 7.2 Hz, H-15), 3.08 (1H, sept, *J* = 6.9 Hz, $(CH_3)_2CH$), 2.91, (1H, dd, *J* = 16.8, 6.2 Hz, H-7β), 2.75 (1H, ddd, *J* = 16.8, 11.3, 7.7 Hz, H-7), 1.39, 1.38 (2×3H, 2d, *J* = 7.2 Hz, H-16, H-17), 1.28 (2×3H, 2d, *J* = 6.9 Hz, $CH(CH_3)_2$), 1.23 (3H, s, H-20), 0.97 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 149.5, 142.6, 131.5, 131.1, 130.4, 119.6 (Ar), 49.7 (C-5), 41.6 (C-3), 39.6 (C-1), 37.9 (C-10), 33.2 (C-4), 33.2 (C-18), 28.6 (C-7), 27.0, 27.0 (C-15,$CH(CH_3)_2$), 25.2 (C-20), 23.0, 22.7 ($CH(CH_3)_2$), 21.5 (C-19), 20.6, 20.6 (C-16, C-17), 19.5 (C-6), 19.4 (C-2); *m/z* 328 (M^+, 45%), 313 (100), 256 (39), 243 (82), 217 (18), 192 (32), 160 (60), 128 (52), 96 (20), 64 (85), 43 (26); HRMS *m/z* calcd for $C_{23}H_{36}O$ (M^+) 328.2766, found 328.2776.

Microbiological testing

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 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.

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