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The Synthesis and Antibacterial Activity of Totarol Derivatives. Part 3: Modification of Ring-B†

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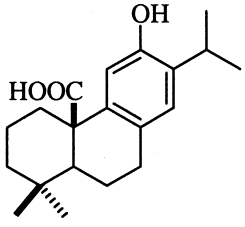
Abstract—Ring-B derivatization of totarol (**1**) afforded the series of compounds **2**–**22** which were screened in vitro against: β-lactamase-positive and high level gentamycin-resistant *Enterococcus faecalis*, penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), and multiresistant *Klebsiella pneumoniae*. Several of the derivatives retained much of the antibacterial activity of totatol against the first three of these organisms (all Gram-positive), but none was more active. The Gram-negative *Klebsiella* was resistant to all compounds examined. Totarol (**1**) was shown to uncouple oxidative phosphorylation in isolated mitochondria at 50 μM. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Lien et al.[1] have shown that lipophilic compounds are trapped in the cell wall lipids of Gram-negative bacteria (lipid content of up to 25% of the dry weight) but are able to pass through Gram-positive bacterial cell walls (0–2.5% lipid content), which results in antibacterial lipophilic alcohols and phenols showing selective activity against Gram-positive bacteria. In keeping with this, Kobayashi et al.[2] have shown that the magnitude and selectivity of the inhibition of Gram-positive bacterial growth by pisiferic acid and its derivatives are a function of the lipophilicity of these compounds.



Pisiferic acid

In a continuation of our investigation into the relationship between the structures of diterpenoid compounds based on the natural product totarol (**1**) and their antibacterial activity,[3,4] we now report a study of the relative antibacterial properties of **1** and of its derivatives **2**–**22** which were obtained by synthetic manipulation of ring-B. Particular interest was taken in the effect of relative lipophilicity on the antibacterial activities.

Results and Discussion

Syntheses of compounds

Firstly, totarol's lipophilicity was modified by hydroxylation of ring-B. Treatment of *O*-methyltotarol with lead tetraacetate in acetic acid by a previously described procedure[3] afforded the 7α-acetate of the starting material and the product formed by replacement of the isopropyl by an acetoxy group in 42 and 16% yield, respectively. Lithium aluminum hydride reduction of the former yielded the 7α-hydroxy derivative **2** (88%) which proved to be unstable to a variety of demethylation protocols (e.g., BBr_3, pyridine hydrochloride).

Synthesis of the corresponding diol **3** was achieved via totaryl acetate,[5] treatment of which with lead tetraacetate in acetic acid gave the previously reported 7α,13-diacetoxy derivative[6] in 44% yield as well as a mixture of other products which ran together on TLC plates. Lithium aluminum hydride reduction of the diacetate provided the desired 7α-hydroxytotarol (**3**),[7] and similar treatment of the mixed fraction afforded triol **7** in 12%

†For part II, see preceding paper.

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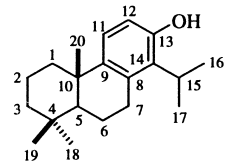
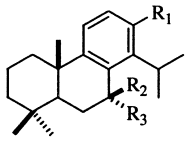
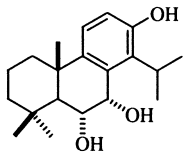


1

	R_1	R_2	R_3
2	OCH_3	H	OH
3	OH	H	OH
4	OH	H	OC_2H_5
5	OH	OH	H
6	OH	OH	CH_3





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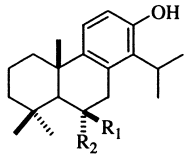
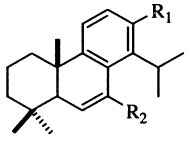
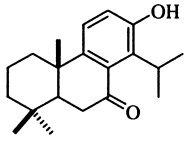


	R_1	R_2
8	H	OH
9	OH	H



	R_1	R_2
10	OH	OCH_3
11	OAc	H
12	OH	H



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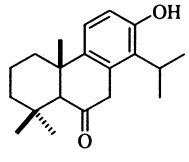

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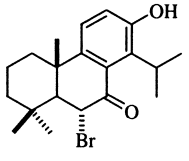

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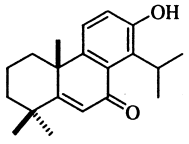

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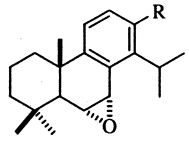
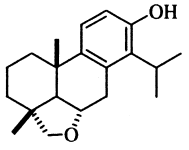


17 R =OAc
18 R = OH



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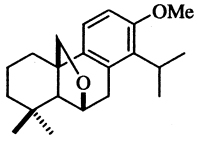

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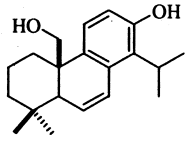

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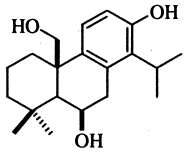


22

yield (based on totaryl acetate). The stereochemistry of this compound was assigned on the basis of H-6 showing 11.6 Hz coupling with H-5 and small coupling with H-7. The former was therefore axial and β and the latter equatorial and also β. In order to prepare the alkene **11**[7] by eliminating acetic acid from the 7α,13-diacetate, the latter was heated in ethanolic HCl under reflux. This process, however, gave three readily separable products: the 7α-ethoxy analogue of the starting material (39%) formed by displacement at the benzylic center and which provided a convenient source of the ethyl ether **4**, the desired alkene **11** (30%) and the product of its deacetylation **12** (26%).

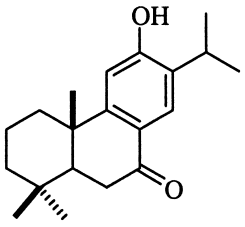
In an attempt to ascertain whether the stereochemistry of the hydroxyl group at C-7 has a bearing on antibacterial activity, the alcohol **5**, the C-7 epimer of **3**, was synthesized in 91% yield by the lithium aluminum hydride reduction of the previously reported 13-*O*-acetyl-7-oxo derivative.[8] The β-stereochemistry at C-7 of compound **5** was confirmed by the characteristic splitting pattern of the C-7-carbinol proton [δ_H 5.15 (t, $J = 8.4$ Hz)] in the ^{1}H NMR spectrum.[9]

Increase in the size of the substituent groups at C-7 should impact on the spatial arrangement of the C-14 isopropyl group and consequently on the phenolic hydroxy group at C-13 since non-bonding *peri*-interactions occur between groups (even hydrogen atoms) at C-7 and the C-14 isopropyl residue.[9] Conceivably biological activity might also be sensitive to such change.[1] Consequently, the 7-oxo-13-*O*-acetyl compound was treated with MeLi in THF and yielded the tertiary alcohol (69%), which on reductive removal of the O-13 acetyl group afforded the phenolic derivative **6**. The presence of the methyl group was evident from the NMR characteristics [δ_H 1.69, 3H (s); δ_C 29.5 (q), 74.9 (s)], and the 7-α-configuration was assigned to the methyl group by analogy, nucleophilic attack at 7-oxo groups of such compounds occurring from the less hindered α-face of the molecules.[10]

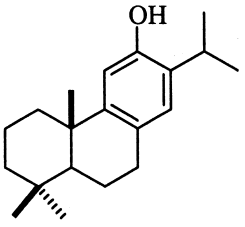
Hydrogenolysis of the epoxyacetate **17**, as previously described,[11] gave 13-acetoxytotara-8,11,13-trien-6α-ol (42%), and lithium aluminum hydride reduction of this product afforded 6α-hydroxytotarol (**8**) (79%), the 6-epimer (**9**) being obtained (69%) by lithium aluminum

hydride reduction of 6-oxototarol (**14**) (see below). The β-stereochemistry at C-6 in **9** was assigned on the basis of the deshielding of the C-10 methyl group resonance [δ_H 1.60 (3H, s)] relative to the corresponding signals in **14** [δ_H 1.33 (3H, s)] and **8** [δ_H 1.17 (3H, s)].

Sugiol, a diterpene closely related to totarol (**1**), has an sp^2 center at C-7 in the form of a carbonyl group and is inactive against Gram-positive bacteria up to 400 μg/mL.[12] In contrast, ferruginol, which is structurally identical to sugiol except that it has a methylene group instead of the carbonyl group at C-7, has been reported as having good antibacterial activity against a variety of Gram-positive bacteria (active at about 8 μg/mL).[3]



Sugiol



Ferruginol

The generality of this loss in antibacterial activity, in consequence of the introduction of an sp^2 center in ring-B, was then studied by way of a range of compounds incorporating one or more sp^2 centers in ring-B of totarol. Such incorporation should lead to: (1) further flattening of the ring-B, (2) the alteration of steric compression between the C-7 methylene group and the C-14 isopropyl group which may have a subsequent effect on the relative spatial arrangement of the isopropyl group and the phenolic group, (3) electronic changes in the aromatic ring, and one or more of these effects could impact on biological properties.

Compound **10** [δ_H 4.97 (1H, d, $J = 3.7$ Hz, H-6), 3.72 (3H, s); δ_C 154.1 (s, C-7), 98.8 (d, C-6), 54.4 (q, CH_3)] was obtained (72%) by lithium aluminum hydride reduction of its *O*-acetate which, in turn, was produced (76%) from the 7-one by treatment with trimethyl orthoformate in the presence of Amberlyst 15 (H^+) resin. Steric impedence apparently inhibited the production of the expected dimethyl acetal.

The $\Delta^{6,7}$ derivatives, compounds **11** and **12**, were synthesized as described above.

7-Oxototarol (**13**), obtained by saponification of its acetate (83%), was shown by X-ray crystallographic analysis (Fig. 1) to have its B-ring in a half-boat conformation as had been proposed earlier by Cutfield et al.[13]

The isomeric 6-oxototarol (**14**) was synthesized by acid-catalyzed elimination of acetic acid from the acetate of **2**, epoxidation of the resulting alkene, and isomerization of the product by treatment with *p*-TsOH in benzene under reflux.[14–16] The *O*-methyl-protected 6-oxo derivative was then demethylated using boron tribromide in

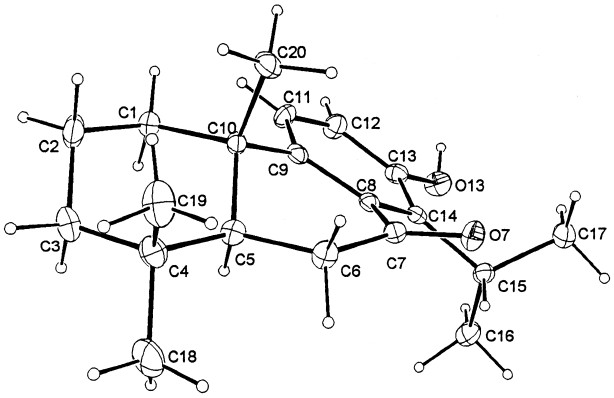


Figure 1.

CH_2Cl_2 to afford **14** in 42% overall yield based on the acetoxy-methoxy starting material.

The 6α-bromo derivative **15**, in which ring-B exists in an approximate boat conformation,[13] was synthesized by bromination of the 13-acetoxy-7-one to give the 6α-bromide (74%), which on saponification afforded **15** in 88% yield. Dehydrobromination of the acetylated bromide using lithium bromide and sodium bicarbonate in DMF gave the corresponding enone (91%) from which the hydroxy-enone **16** was obtained in 89% yield by deacetylation.

The incorporation of fused and bridging heterocyclic ring systems onto the totarol skeleton was also investigated. Treatment of the acetylated alkene **11** with mCPBA gave the previously described epoxide **17**[11] (92%) which, on saponification, afforded the deprotected derivative **18** (82%).

The introduction of fused oxolane rings, by remote functionalization of either of the β-methyl groups at C-4 or C-10 in similar diterpenoid systems, has been described previously.[11,14] Treatment of 6α-hydroxytotaryl acetate, obtained by the hydrogenation of epoxide **17**, with lead tetraacetate and iodine in benzene gave the 6α,18-epoxy derivative (96%)[10] from which compound **19** was derived (96%) by reductive deacetylation. The intermediate acetate appears to have been incorrectly assigned as the 6β,19-epoxy derivative on the basis of ^{1}H NMR data.[11] The absence, in the ^{13}C NMR spectrum, of the characteristic resonance of C-18 (δ_C 33.0) and the presence of that of C-19 [δ_C 19.1 (q)] indicates the 6α,18-epoxy derivative had been formed.

Similar oxidative treatment of the 13-methyl ether of the 6β-alcohol **9**, produced by lithium aluminum hydride reduction of the methyl ether of ketone **14**, afforded the 6β,20-epoxy derivative **20** in 90% yield. The structure of **20** was established by the presence in its ^{1}H and ^{13}C NMR spectra of resonances characteristic of a cyclic ether [δ_H 4.57 (1H, brs, H-6), 4.01, 3.73 (2H, 2d, $J = 7.2$ Hz, H-20ab); δ_C 78.4 (t, C-20), 77.6 (d, C-6)] and by the absence of the signal due to the 10β-methyl group and the presence of those characteristic of the C-18 and C-19 methyl groups.

Attempts to deprotect compound **20** with BBr_3 and leave the ether ring intact were unsuccessful, and two new compounds, diol **21** (39%) and triol **22** (44%), were isolated. The structure of 13,20-dihydroxytotara-6,8,11,13-tetraene (**21**) was confirmed by the presence in its ^1H and ^{13}C NMR spectra of signals characteristic of $\Delta^{6,7}$-alkene group [δ_H 6.89 (1H, dd, $J=10.1$, 3.1 Hz, H-7), 5.96 (1H, dd, $J=10.1$, 3.1 Hz, H-6); δ_C 130.1 (d, C-6), 124.3 (d, C-7)] and a hydroxylmethyl group at C-10 [δ_H 3.62, 3.38 (2H, 2t, $J=6.4$ Hz, H-20ab); δ_C 59.8 (t)]. The structure of 6β,13,20-trihydroxytotara-8,11,13-triene (**22**) was confirmed by the presence in its ^1H and ^{13}C NMR spectra of signals characteristic of a 6β-hydroxyl group [δ_H 5.29 (1H, d, $J=2.1$ Hz, H-6α); δ_C 66.9 (t)] and a hydroxylmethyl group at C-10 [δ_H 4.27 (1H, d, $J=8.2$ Hz, H-20a), 2.80 (1H, dd, $J=8.2$, 1.6 Hz, H-20b); δ_C 67.6 (t)]. The large difference in the NMR shifts of the C-20 protons is likely to be due to restricted rotation about the C-10,C-20 bond caused by intramolecular hydrogen bonding between the *cis*-related hydroxyl groups at C-6 and C-20, the former being axial and the latter being part of the axial hydroxylmethyl substituent at C-10.

In vitro antibacterial screening

Listed in Table 1 are the minimum inhibitory concentrations of compounds **2**–**22** together with those of the reference compound totarol (**1**) when screened 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 multi-resistant *Klebsiella pneumoniae* at the concentrations 2, 8 and 32 μg mL^{-1}.

As with totarol itself, none of the derivatives displayed activity against the Gram-negative organism *Klebsiella pneumoniae*, up to the highest concentration tested (32 μg mL^{-1}). The key conclusions are:

1. a trend was observed between antibacterial activity and lipophilicity since the 7α-ethoxy derivative **4** exhibited antibacterial activity comparable with that of **1**, as did **6**, whereas the diol analogues **3** and **5** showed only weak inhibitory activity, and the least active compound was the trihydroxy derivative **7**;
2. the introduction, into ring-B, of a double bond, i.e., two new sp^2 centers as in **10** and **12** had little or no effect on antibacterial activity relative to totarol (**1**) whereas, as in the case of ferruginol and sugiol, the 7-oxo derivative of totarol, compound **13**, was inactive despite the fact that the other 7-oxo derivatives **15** and **16** and the 6-oxo derivative **14** exhibited persisting degrees of activity;
3. the addition of a fused and bridging heterocyclic ring system into totarol (**1**) was of no assistance; however, compounds **21** and **22** were both active, the more lipophilic, **21**, being slightly the more potent;
4. again,[3] *O*-methylation or acetylation of the phenolic hydroxyl group (compounds **2**, **11**, **17** and **20**) virtually eliminated activity;
5. also in keeping with earlier findings,[3] activity against *Streptococcus pneumoniae* was more easily retained than against the other two Gram-positive strains.

The interaction of totarol (1) and 7-oxototarol (13) with mitochondria

Some studies on the mechanism of the antibacterial activity of lipophilic phenolic compounds such as totarol have implicated cell wall biosynthesis as the likely target. There is another possible mechanism,

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	> 32	> 32
3	32 (106)	8 (26)	32 (106)	> 32
4	8 (24)	2 (6)	2 (6)	> 32
5	32 (106)	2 (7)	> 32	> 32
6	8 (25)	2 (6)	8 (25)	> 32
7	> 32	32 (100)	32 (100)	> 32
8	32 (106)	2 (7)	> 32	> 32
9	32 (106)	8 (27)	8 (27)	> 32
10	8 (25)	2 (6)	8 (25)	> 32
11	> 32	> 32	> 32	> 32
12	8 (28)	2 (7)	2 (7)	> 32
13	> 32	> 32	> 32	> 32
14	8 (27)	8 (27)	8 (27)	> 32
15	8 (21)	2 (5)	8 (21)	> 32
16	> 32	8 (27)	> 32	> 32
17	32 (93)	32 (93)	> 32	> 32
18	> 32	32 (11)	32 (11)	> 32
19	> 32	8 (27)	> 32	> 32
20	> 32	> 32	> 32	> 32
21	32 (107)	8 (27)	32 (107)	> 32
22	> 32	8 (25)	8 (25)	> 32

[a]Minimum inhibitory concentrations.

however, that has yet to be examined: totarol and its analogues could be acting by disrupting bacterial energy metabolism. This could be by three possible means: either by acting as specific proton ionophores, thus uncoupling oxidative phosphorylation, by non-specifically disrupting bacterial membrane structure or by specifically inhibiting an enzyme in energy metabolism, in much the same way as cyanide does.

Oxidative phosphorylation, which is the major process by which cells synthesize ATP, requires proton translocation across the lipid bilayer membrane which surrounds the cell. In this way cells maintain a proton electrochemical gradient across their membrane which is then used as an energy source to drive ATP synthesis. A large number of lipophilic and weakly basic phenolate compounds, called uncouplers, prevent the formation of this proton electrochemical potential gradient by crossing the lipid bilayer, bringing protons from regions of high potential to regions of low potential. As a result, the cell speeds up respiration in an attempt to maintain the proton electrochemical potential across its membrane. Uncoupling prevents the cells from synthesizing ATP and thus leads to cells dying due to energy depletion. General membrane disruption also has a similar result. This can occur when large amounts of hydrophobic/amphipathic compounds partition into the hydrophobic lipid bilayer and thus disrupt its structure, making it "leaky" to protons and thus impossible for the cell to maintain a proton electrochemical potential. In addition, this disruption of membrane structure may also directly inhibit the membrane-embedded enzymes necessary for energy metabolism. Neither of these mechanisms is particularly appealing for an antibiotic if it is to have therapeutic applications as the energy factories in mammalian cells are mitochondria, small intracellular vesicles surrounded by a lipid bilayer. Any compound that uncoupled or disrupted this bilayer would be potentially toxic. For example, the potent uncoupler 2,4-dinitrophenol was once used as a slimming agent but was later withdrawn because of its toxicity.

For these reasons, we chose to specifically examine these possible mechanisms of action, using isolated rat liver mitochondria. Mitochondrial respiration is a good model for that in Gram-positive bacteria, and at the same time results might provide an indication of likely mammalian sensitivity to the new class of antibiotics under study. As test compounds we chose totarol (**1**), which is antibacterial at 2 μg mL^{-1}, and 7-oxototarol (**13**), which is inactive against the three strains of Gram-positive bacteria at 32 μg mL^{-1}. We considered that these compounds are likely to be not too dissimilar with regard to lipophilicity and basicity and, therefore, would disrupt biological membranes and act as classical uncouplers to a similar extent.

The first experiment looked at the ability of the two diterpenoid compounds to interfere with coupled mitochondrial respiration. Using succinate as the respiratory substrate, the results shown in Figure 2 were obtained. At 10 μM, neither compound **1** nor **13** had an effect on

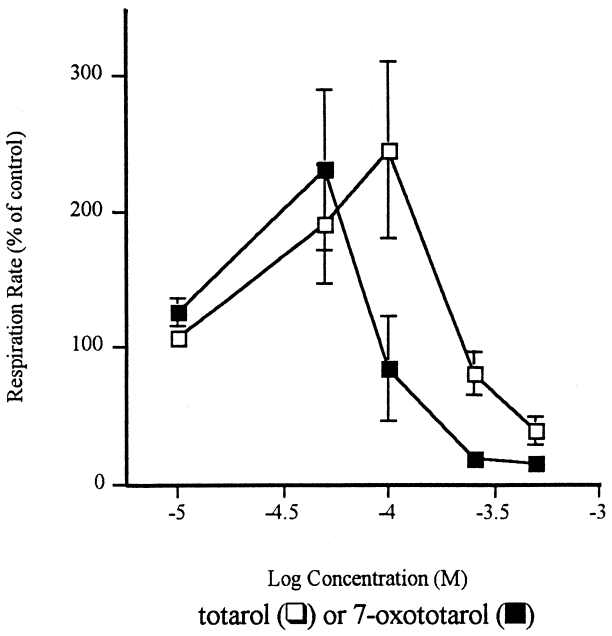


Figure 2.

respiration rate, but at 50 μM both accelerated respiration about 2-fold, and at higher concentrations both shut down respiration. These results are consistent with both diterpenoids being able to increase the proton permeability of the membrane, either by acting as classical uncouplers or by disrupting the lipid bilayer structure. This increase in respiration occurs as the respiratory chain speeds up in an effort to compensate for the increased proton leakage through the membrane. However, this increase in proton leakage only occurred at concentrations 10- to 100-fold higher than required for complete antibacterial activity and is therefore unlikely to contribute to the antibacterial effect.

A second experiment (data not shown) confirmed that the initial stimulation of respiration caused by these compounds was due to increasing the proton leakage through the mitochondrial membrane, and not to a specific interaction with the respiratory chain. To do this, combinations of glutamate and malate, or ascorbate and TMPD, were used as respiratory substrates. These substrates were chosen because they utilize different sections of the respiratory chain from succinate. The results were very similar to those found with succinate as substrate, with stimulation of respiration occurring in the presence of 50–100 μM of the diterpenoid compounds. This again is consistent with both compounds stimulating respiration non-specifically by increasing the proton permeability of the membrane.

A third set of experiments examined respiration in the presence of a known potent uncoupler, FCCP, together with each of the various respiratory substrates mentioned above, separately. The concentration of FCCP (333 nM) was selected to be sufficient to completely uncouple the mitochondria. This was done so that any stimulation of respiration by uncoupling by the

diterpenoid compounds would be eliminated and only their inhibitory effects would remain. Under these conditions, one would expect that, if the diterpenoid compounds were acting as membrane disrupters (or indeed as specific inhibitors of any other part of the respiratory chain), a substantially enhanced inhibition of respiration would be seen. Results using succinate as a substrate are shown in Figure 3. Indeed, both totarol (**1**) and 7-oxototarol (**13**) were able to progressively inhibit respiration at 50 μM and above, but not at 10 μM or below. Similar results were obtained with glutamate/malate as respiratory substrates, but with ascorbate/TMPD the inhibition of respiration brought about with either of the diterpenoids was less (data not shown). This inhibition is probably due to non-specific disruption of the lipid bilayer interfering with the activity of the oxidizing enzymes in the mitochondrial membrane.

Finally, the effect of the diterpenoid compounds upon phosphorylating respiration through a specific inhibition of ATP-synthase was probed, using each of the various respiratory substrates mentioned above, separately. No evidence for specific inhibition of this enzyme was found.

In conclusion, it is apparent that both diterpenoids **1** and **13** interfere modestly with mitochondrial respiration, by increasing the proton permeability of the membrane, either by acting as classic uncouplers, by general membrane disruption, or both. There are two strong arguments against these mechanisms being the cause of the antibacterial activity of totarol and its analogues, however. Firstly, effects upon respiration only became evident at concentrations 10- to 100-fold higher than required for complete antibacterial activity. Secondly, and most persuasively, 7-oxototarol (**13**) was slightly more potent than totarol (**1**) in affecting respiration and yet it is considerably less potent as an antibacterial agent. The relative insensitivity of mitochondrial respiration towards totarol (**1**) at concentrations above its in vitro antibacterial MIC removes one possible impediment to the use of this class of compounds as therapeutics.

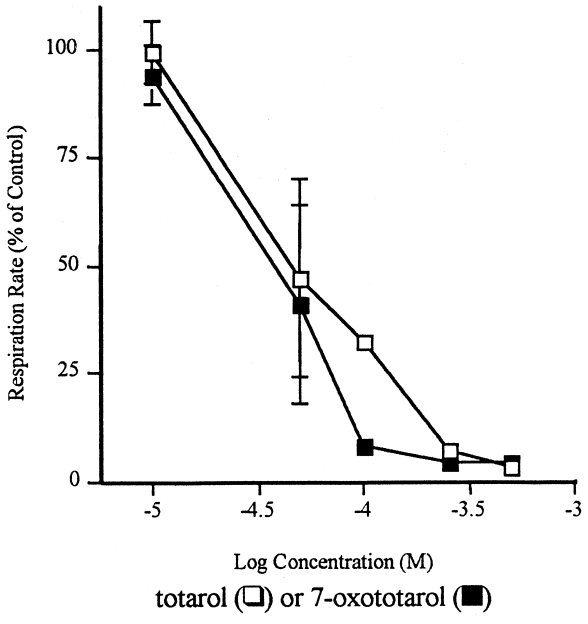


Figure 3.

X-ray single crystal analysis

The ORTEP diagram of 7-oxototarol (**13**) is shown in Figure 1. The independent molecules are hydrogen bonded through the hydroxyl hydrogen atom on O-13 and the carbonyl oxygen atom (O-7) of an adjacent molecule forming chains along the *z* axis: O-7···H(O-13) = 1.96 (4) Å; O-7···O-13 = 2.731(4) Å; O-13···H(O-13)···O-7 = 171(4)° (O-7 translated by: $1/2-x$, $1-y$, $z-1/2$). Ring A adopts a slightly distorted chair conformation with best mean plane through C-1, C-10, C-3, and C-4 (± 0.008 Å) with C-2, C-5, respectively, −0.688(6) and +0.577(5) Å out of plane; the slight distortion is shown in the Q(2),Π[17] values of 0.069(4) Å, 7.4(4)° compared with a pure chair 0 Å, 0°. Ring-B adopts a twist boat [Π, > 73.4(4), 333.6(4)° compared with theoretical 90, 330°];[17] the twist results in the carbonyl atoms C-7, O-7 being 0.316(5), 1.043(6) Å respectively from the plane of ring-C, in the direction of the 9-methyl group C-20. Molecular modeling studies (vapor phase)[18] indicated that the alternative conformation in which the carbonyl group is twisted towards the α-face of the molecule is less stable by 0.7 kcal mol^{-1}. This suggests that the twist direction is a phenomenon associated with the molecule itself and not determined by intermolecular hydrogen bonding in the crystal.

The aromatic ring-C is essentially planar with average deviations of 0.024(3) Å, and O-13 and C-10 only 0.036(6) and 0.049(6) Å from the mean plane respectively. Atom C-15 is out of plane by 0.164(6) Å *anti* to O-7. The aromatic ring plane does not exactly bisect the isopropyl carbon atoms (C-16, C-15, C-17) angle, C-16 and C-17 being +1.445(7), −1.064(7) Å, respectively, from the plane. The overall geometry of the molecule is very similar to that of methyl 6α-bromo-13-isopropyl-7-oxopodocarpa-8,11,13-trien-15-oate,[13] with a slightly flatter ring-B (dihedral angle C-6–C-7–C-8–C-9 = 33.7(5)°) being the only concession to the bulky bromine atom.

Experimental

Syntheses

General methods. ^{1}H and ^{13}C NMR data were recorded on a Bruker AC300 spectrometer and assignments were made with the assistance of DEPT and COSY experiments, the infra-red spectra were measured on a Perkin Elmer 1600 series FTIR spectrometer, elemental analyses were obtained on 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 60 PF_{254} silica gel-coated aluminum sheets. All purifications were by column chromatography which was performed using Merck Kieselgel S silica gel. Solvents were dried and purified before use according to standard procedures.[19] "Petrol" refers to the fraction of petroleum ether boiling between 60 and 80 °C.

13-Methoxytotara-8,11,13-trien-7α-ol (2). Lithium aluminum hydride (20 mg) was added to a stirred solution of 7α-acetoxy-13-methoxytotara-8,11,13-triene[3] (40 mg, 0.11 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 30 min, quenched with water (0.04 mL), NaOH (15% aqueous, 0.04 mL), and water (0.12 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave **2** (31 mg, 88%); mp 83 °C; ν_{max} 3448 (OH), 1640 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.12, 6.83 (2H, 2d, J = 8.8 Hz, H-11, H-12), 5.01 (1H, br s, H-7β), 3.78 (3H, s, OCH_3), 3.53 (1H, sept, J = 6.9 Hz, H-15), 1.37, 1.33 (2×3H, 2d, J = 6.9 Hz, H-16, H-17), 1.13 (3H, s, H-20), 1.02 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 157.3, 142.8, 135.8, 134.5, 128.7, 112.5 (Ar), 65.6 (C-7), 55.1 (OCH_3), 44.1 (C-5), 41.5 (C-3), 39.1 (C-1), 38.0 (C-10), 33.5 (C-18), 33.1 (C-4), 28.9 (C-6), 28.0 (C15), 24.8 (C-20), 21.7 (C-19), 21.2, 21.2 (C-16, C-17), 19.4 (C-2); m/z 316 (M^+, 25%), 298 (100), 241 (82), 213 (61), 171 (45), 115 (13), 69 (14); HRMS calcd for $C_{21}H_{32}O_2$ 316.2402, found 316.2399.

Lead tetraacetate oxidation of 13-acetoxytotara-8,11,13-triene. Lead tetraacetate (15 g, 34 mmol) was added to a stirred solution of the acetate (7.5 g, 23 mmol) in acetic acid (100 mL) and the solution was heated to 100 °C under argon. The mixture was stirred for 2 h at 100 °C, another portion of lead tetraacetate (7.5 g, 17 mmol) was added and stirring was continued for a further 2 h at this temperature. Water (1 L) was added and the mixture was extracted with ethyl acetate (3×150 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 (50:1) as the eluent gave 7α,13-diacetoxy-totara-8,11,13-triene[6] (3.9 g, 44%); mp 155 °C; ^1H NMR ($CDCl_3$) δ 7.13, 6.90 (2H, 2d, J = 8.8 Hz, H-11, H-12), 6.01 (1H, br s, H-7β), 2.86 (1H, sept, J = 7.0 Hz, H-15), 2.24, 1.97 (2×3H, 2s, $COCH_3$), 1.19, 1.15 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.09 (3H, s, H-20), 0.83 (3H, s, H-18), 0.82 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 170.5, 169.9 ($COCH_3$), 69.3 (C-7), 44.6 (C-5), 41.5 (C-3), 39.2 (C-1), 39.0 (C-10), 33.3 (C-18), 33.3 (C-4), 28.0 (C-15), 26.5 (C-6), 24.4 (C-20), 21.7, 21.6 (C-16, C-17), 21.3 (C-19), 20.9, 20.9 ($COCH_3$), 19.7 (C-2). This was followed by an inseparable mixture of compounds from which compound **7** was obtained (see below).

Totara-8,11,13-triene 7α,13-diol (3).[7] Lithium aluminum hydride (100 mg) was added cautiously to a stirred solution of 7α,13-diacetoxytotara-8,11,13-triene (200 mg, 0.52 mmol) in THF (10 mL) under argon at room temperature and stirring was continued for 30 min. The reaction was quenched with water (0.1 mL), NaOH (15%, 0.1 mL), and water (0.3 mL), filtered, and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (4:1) as the eluent gave diol **3** (142 mg, 90%); mp 216–218 °C, lit.[7] 215–217.5 °C; ^1H NMR ($CDCl_3$) δ 7.03, 6.64 (2H, 2d, J = 8.6 Hz, H-11, H-12), 5.03 (1H, br s, H-7β), 4.89 (1H, s, OH), 3.58 (1H, sept, J = 7.1 Hz, H-15), 1.44, 1.40 (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 1.13 (3H, s, H-20), 1.00 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 153.0, 142.9, 134.6, 133.1, 123.5, 117.2 (Ar), 65.7 (C-7), 44.2 (C-5), 41.5 (C-3), 39.2 (C-1), 38.4 (C-10), 33.1 (C-18), 32.9 (C-4), 29.1 (C-6), 27.7 (C-15), 24.6 (C-20), 21.7 (C-19), 21.0, 20.8 (C-16, C-17), 19.5 (C-2).

Ethanolysis of 7α,13-diacetoxytotara-8,11,13-triene. Dilute hydrochloric acid (1 mL, 1.2 M) was added dropwise to a stirred solution of the diacetate (330 mg, 0.92 mmol) in ethanol (20 mL) and the reaction mixture was heated under reflux for 1.5 h. The volatiles were removed under reduced pressure and the residue was resuspended in ethyl acetate (100 mL), washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:CH_2Cl_2 (1:1) as the eluent gave the following in order of elution: 13-acetoxy-7α-ethoxytotara-8,11,13-triene as an oil (133 mg, 39%); ^1H NMR ($CDCl_3$) δ 7.17, 6.92 (2H, 2d J = 8.8 Hz, H-11, H-12), 4.51 (1H, br s, H-7β), 3.79, 3.47 (2×1H, 2dt, J = 8.6, 7.0 Hz, OCH_2), 3.20 (1H, sept, J = 7.0 Hz, H-15), 2.33 (3H, s, $COCH_3$), 1.37, 1.28 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.27 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.19 (3H, s, H-20), 1.00 (3H, s, H-18), 0.96 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 169.5 ($COCH_3$), 148.0, 147.6, 138.7, 133.6, 123.6, 123.4 (Ar), 73.4 (C-7), 63.3 (OCH_2CH_3), 43.6 (C-5), 41.0 (C-3), 38.7 (C-1), 38.7 (C-10), 32.9 (C-4), 32.8 (C-18), 27.5 (C-15), 24.2 (C-20), 22.8 (C-6), 21.7 (C-19), 21.6, 21.5 (C-16, C-17), 21.1 ($COCH_3$), 19.4 (C-2), 15.7 (CH_2CH_3); m/z 372 (M^+, 3%), 326 (72), 284 (87), 269 (100), 227 (31), 199 (32), 185 (14), 173 (16), 157 (17), 69 (17), 55 (18), 43 (77); HRMS m/z calcd for $C_{24}H_{36}O_3$ (M^+) 372.2664, found 372.2661.

This was followed by 13-acetoxytotara-6,8,11,13-tetraene (**11**)[7] as a solid (91 mg, 30%); mp 137–139 °C, lit.[7] 138–140 °C; ^1H NMR ($CDCl_3$) δ 7.04, 6.83 (2H, J = 8.4 Hz, H-11, H-12), 6.88 (1H, dd, J = 10.1, 3.0 Hz, H-7), 6.07 (1H, dd, J = 10.1, 3.0 Hz, H-6), 3.37 (1H, sept, J = 7.2 Hz, H-15), 2.32 (3H, s, $COCH_3$), 1.32, 1.29 (2×3H, 2d, J = 7.2 Hz, H-16, H-17), 1.06 (3H, s, H-20), 1.06 (3H, s, H-19), 0.98 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 169.9 ($COCH_3$), 147.3, 146.8, 134.5, 132.1 (Ar), 131.0 (C-6), 124.0 (C-7), 121.9, 120.7 (Ar), 49.8 (C-5), 41.0 (C-3), 38.6 (C-10), 36.6 (C-1), 32.8 (C-4), 32.6 (C-18), 27.4 (C-15), 22.5 (C-20), 21.8 (C-19), 21.6, 21.4 (C-16, C-17), 20.1 ($COCH_3$), 19.1 (C-2).

This was followed by totara-6,8,11,13-tetraen-13-ol (**12**)[7] as an oil (67 mg, 26%); ^1H NMR ($CDCl_3$) δ 6.89 (1H, dd, J = 10.1, 3.0 Hz, H-7), 6.90, 6.56 (2H, 2d, J = 8.3 Hz, H-11, H-12), 6.06 (1H, dd, J = 10.1, 3.0 Hz,

H-6), 3.49 (1H, sept, $J=7.1$ Hz, H-15), 1.42, 1.39 (2×3H, 2d, $J=7.1$ Hz, H-16, H-17), 1.06 (3H, s, H-20), 1.04 (3H, s, H-19), 0.99 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 152.3, 142.1, 131.8 (Ar), 130.9 (C-6), 129.6 (Ar), 124.3 (C-7), 120.5, 115.0 (Ar), 50.2 (C-5), 41.1 (C-3), 38.4 (C-10), 36.8 (C-1), 32.7 (C-4), 32.6 (C-18), 27.4 (C-15), 22.4 (C-20), 21.4 (C-19), 21.1, 20.4 (C-16, C-17), 19.2 (C-2).

7α-Ethoxytotara-8,11,13-trien-13-ol (4). Lithium aluminum hydride (60 mg) was added cautiously to a stirred solution of 13-acetoxy-7α-ethoxytotara-8,11,13-triene (120 mg, 0.32 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 30 min, quenched with water (0.06 mL), NaOH (15%, 0.06 mL), and water (0.18 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave as an oil **4** (92 mg, 87%); ν_{max} 3369 (OH), 1584 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.99, 6.56 (2H, 2d $J=8.6$ Hz, H-11, H-12), 4.92 (1H, s, OH), 4.51 (1H, t, $J=2.5$ Hz, H-7β), 3.80, 3.48 (2H, 2dq $J=8.6$, 7.0 Hz, OCH_2CH_3), 3.18 (1H, sept, $J=7.0$ Hz, H-15), 1.44, 1.40 (2×3H, 2d, $J=7.0$ Hz, H-16, H-17), 1.27 (3H, t, $J=7.0$ Hz, OCH_2CH_3), 1.15 (3H, s, H-20), 0.99 (3H, s, H-18), 0.95 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.7, 143.0, 133.3, 133.2, 123.3, 117.1 (Ar), 73.7 (C-7), 63.4 (OCH_2CH_3), 43.8 (C-5), 41.1 (C-3), 38.9 (C1), 38.3 (C-10), 32.9 (C-4), 32.9 (C-18), 27.8 (C-15), 24.5 (C-20), 22.9 (C-6), 21.7 (C-19), 20.8, 20.5 (C-16, C-17), 19.5 (C-2), 15.8 (CH_2CH_3); m/z 330 (M^+, 11%), 284 (68), 269 (100), 227 (46), 199 (52), 157 (37), 43 (32); HRMS m/z calcd for $C_{22}H_{34}O_2$ 330.2559 (M^+), found 330.2554.

13-Acetoxytotara-8,11,13-trien-7-one.[8] *N*-Bromosuccinimide (16.3 g, 75 mmol) was added to a stirred solution of totaryl acetate (10 g, 30 mmol) and $CaCO_3$ (9 g, 60 mmol) in THF (200 mL) and water (5 mL) at room temperature, and the resulting reaction mixture was stirred for a further 1 h while being irradiated with visible light.[20] The mixture was filtered, the filtrate was concentrated in vacuo, water (500 mL) was added and the resulting aqueous suspension extracted into ethyl acetate (3×100 mL). The combined organic layer was washed with aqueous $Na_2S_2O_3$ (3×50 mL), brine (3×50 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave the title ketone (7.4 g, 71%); mp 170–171 °C, lit.[8] 169–170 °C; ^{1}H NMR ($CDCl_3$) δ 7.21, 7.09 (2H, 2d, $J=8.6$ Hz, H-11, H-12), 3.73 (1H, sept, $J=7.0$ Hz, H-15), 2.35 (3H, s, $COCH_3$), 1.38, 1.23 (2×3H, d, $J=7.0$ Hz, H-16, H-17), 1.14 (3H, s, H-20), 1.04 (3H, s, H-18), 0.94 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 202.1 (C-7), 169.5 ($COCH_3$), 153.5, 148.2, 139.9, 133.9, 127.1, 121.3 (Ar), 47.5 (C-5), 41.4 (C-3), 38.7 (C-1), 38.4 (C-6), 38.2 (C-10), 33.2 (C-4), 32.1 (C-18), 27.9 (C-15), 22.8 (C-20), 21.6, 21.4, 21.3, 21.3 (C-19, C-16, C-17, $COCH_3$), 18.8 (C-2).

Totara-8,11,13-triene-7β,13-diol (5).[13] Lithium aluminum hydride (50 mg) was added portionwise to a stirred solution of the 13-*O*-acetyl-7-one (see above, 100 mg, 0.3 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.05 mL), NaOH (15%, 0.05 mL), and water (0.15 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (10:1) as the eluent gave **5** as an oil (74 mg, 82%); ^{1}H NMR ($CDCl_3$) δ 6.98, 6.62 (2H, 2d $J=8.6$ Hz, H-11, H-12), 5.37 (1H, s, OH), 5.15 (1H, t, $J=8.4$ Hz, H-7α), 3.55 (1H, sept, $J=7.1$ Hz, H-15), 1.49, 1.37 (2×3H, 2d, $J=7.1$ Hz, H-16, H-17), 1.29 (3H, s, H-20), 0.96 (2×3H, s, H-18, H-19); ^{13}C NMR ($CDCl_3$) δ 153.4, 144.2, 136.2, 133.4, 122.7, 116.5 (Ar), 68.4 (C-7), 48.8 (C-5), 41.5 (C-3), 39.9 (C-1), 37.9 (C-10), 33.1 (C-4), 32.9 (C-18), 30.9 (C-6), 28.7 (C-15), 24.8 (C-20), 21.5 (C-19), 21.0, 20.9 (C-16, C-17), 19.3 (C-2).

13-Acetoxy-7α-methyltotara-8,11,13-trien-7β-ol. The 13-acetoxy-7-one (see above) (300 mg, 0.88 mmol) was dissolved in THF (10 mL) and cooled to −78 °C with stirring. MeLi (1.5 mL, 1.0 M in diethyl ether) was added dropwise to the resulting soultion and after 10 min the reaction mixture was allowed to warm to ambient temperature and then quenched with aqueous NH_4Cl (10%, 100 mL). The resulting aqueous layer was extracted with ethyl acetate (3×50 mL) and the combined organic phases were washed with brine (3×20 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave the title tertiary alcohol (210 mg, 69%); mp 122 °C; ν_{max} 3486 (OH), 1754 (CO), 1194 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.12, 6.87 (2H, dd, $J=8.8$ Hz, H-11, H-12), 4.30 (1H, sept, $J=6.9$ Hz, H-15), 2.31 (3H, s, $COCH_3$), 1.66 (3H, s, CH_3), 1.29, 1.28 (2×3H, 2d, $J=7.0$ Hz, H-16, H-17), 1.25 (3H, s, H-20), 0.98 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 169.7 ($COCH_3$), 148.9, 147.2, 140.3, 139.0, 123.6, 123.1 (Ar), 74.6 (C-7), 48.0 (C-5), 41.1 (C-3), 40.6 (C-1), 39.6 (C-6), 39.6 (C-10), 32.8 (C-4), 32.6 (C-18), 29.7 (CH_3), 27.7 (C-15), 25.2 (C-20), 21.6 ($COCH_3$), 21.6 (C-19), 21.3, 20.8 (C-16, C-17), 19.4 (C-2); m/z 358 (M^+, 3%), 340 (69), 283 (100), 267 (15), 213 (42), 187 (32), 171 (19), 91 (10), 69 (23); HRMS m/z calcd for $C_{23}H_{34}O_3$ (M^+) 358.2508, found 358.2506.

7α-Methyltotara-8,11,13-triene-7β,13-diol (6). Lithium aluminum hydride (100 mg) was added portionwise to a stirred solution of 13-acetoxy-7α-methyltotara-8,11,13-trien-7β-ol (100 mg, 0.3 mmol) in THF (10 mL) under argon at room temperature. The mixture was stirred for 2 h, quenched with water (0.1 mL), NaOH (15%, 0.1 mL), and water (0.3 mL), filtered, and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave diol **6** (85 mg, 96%); mp 160 °C; ν_{max} 3461, 3224 (OH), 1583 (C=C), 1190 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.99, 6.62 (2H, 2d $J=8.6$ Hz, H-11, H-12), 5.40 (1H, s, OH), 4.26 (1H, sept, $J=7.1$ Hz, H-15), 1.69 (3H, s, CH_3), 1.42 (2×3H, 2d, $J=7.1$ Hz, H-16, H-17), 1.24 (3H, s, H-20), 0.99 (3H, s, H-18), 0.93 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 153.9, 142.2, 139.9, 133.1, 123.5, 116.4 (Ar), 74.9 (C-7), 48.1 (C-5), 41.1 (C-3), 40.5 (C-1), 39.7 (C-6), 39.1 (C-10), 32.6 (C-4), 32.6 (C-18), 29.5 (CH_3), 28.0 (C-15), 25.3 (C-20), 21.5 (C-19), 20.5, 20.1 (C-16, C-17), 19.3 (C-2); m/z 316 (M^+, 10%), 298 (85), 283 (100), 213 (44),

171 (16), 98 (11), 83 (85); HRMS calcd for $C_{21}H_{32}O_2$ 316.2402, found 316.2402.

Totata-8,11,13-trien-6α,7α,13-triol (7). Lithium aluminum hydride (150 mg) was added cautiously to a stirred solution of the mixed fraction derived by lead tetraacetate treatment of totaryl acetate (see above) (400 mg) in THF (20 mL) under argon at room temperature. The reaction mixture was stirred for 30 min, quenched with water (0.15 mL), NaOH (15%, 0.15 mL), and water (0.45 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave the title triol **7** as an oil (131 mg); ν_{max} 3412 (OH), 1630 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.94, 6.62 (2H, 2d $J=8.6$ Hz, H-11, H-12), 5.57 (1H, s, OH), 4.93 (1H, br s, H-7β), 4.31 (1H, d, $J=11.6$ Hz, H-6β), 3.47 (1H, sept, $J=7.0$ Hz, H-15), 1.46, 1.35 (2×3H, 2d, $J=7.0$ Hz, H-16, H-17), 1.30 (3H, s, H-20), 1.21 (3H, s, H-18), 1.16 (3H, s, H19). δ_C ($CDCl_3$) 153.7, 143.2, 134.6, 133.4, 122.3, 116.9 (Ar), 78.2, 77.2 (C6, C7), 53.3 (C5), 43.6 (C-3), 40.3 (C-1), 39.4 (C-10), 36.1 (C-18), 33.6 (C-4), 28.9 (C-15), 26.2 (C-20), 22.0 (C-19), 21.0, 21.0 (C-16, C-17), 19.2 (C-2); *m/z* 318 (M^+, 23%), 300 (76), 267 (30), 243 (52), 215 (70), 202 (28), 187 (22), 173 (25), 69 (42), 55 (22), 41 (28); HRMS *m/z* calcd for $C_{20}H_{30}O_3$ (M^+) 318.2195, found 318.2190.

13-Acetoxytotara-8,11,13-trien-6α-ol.[11] A mixture of the epoxyacetate **17**[11] (1.3 g, 3.8 mmol), Pd/C (250 mg) and isopropanol (20 mL) was stirred at room temperature under an atmosphere of hydrogen for 14 h. The Pd/C was removed by filtration through Celite and the volatiles were removed in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave the title alcohol (630 mg, 49%); mp 136–138 °C, lit.[11] 136–139 °C; ^1H NMR ($CDCl_3$) δ 7.08, 6.80 (2H, 2d, $J=8.6$ Hz, H-11, H-12), 4.33 (1H, m, H-6), 3.30 (1H, sept, $J=7.2$ Hz, H-15), 3.11 (2H, m, H-7a,b), 2.30 (3H, s, $COCH_3$),1.28, 1.28 (2×3H, 2t, $J=7.2$ Hz, H-16, H-17), 1.17 (3H, s, H-20), 1.11 (2×3H, 2s, H-18, H-19); ^{13}C NMR ($CDCl_3$) δ 169.8 ($COCH_3$), 147.6, 147.1, 136.5, 133.1, 121.3, 120.8 (Ar), 68.6 (C-6), 57.0 (C-5), 42.7 (C-3), 39.6 (C-1), 38.8 (C-10), 37.0 (C-7), 34.8 (C-18), 33.8 (C-4), 27.2 (C-15), 23.0 (C-20), 22.2 (C-19), 21.3, 21.1 (C-16, C-17), 21.3 ($CO\mathit{C}H_3$), 18.9 (C-2).

Totara-8,11,13-triene-6α,13-diol (8). Lithium aluminum hydride (50 mg) was added portionwise to a stirred solution of 13-acetoxytotara-8,11,13-trien-6α-ol[11] (100 mg, 0.30 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.05 mL), NaOH (15%, 0.05 mL), and water (0.15 mL), filtered and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (10:1) as the eluent gave diol **8** (86 mg, 98%); mp 177 °C; ν_{max} 3372 (OH), 1589 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.92, 6.55 (2H, 2d $J=8.4$ Hz, H-11, H-12), 4.81 (1H, s, OH), 4.35 (1H, m, H-6β), 3.39 (1H, sept, $J=6.9$ Hz, H-15), 3.22, 3.00 (2H, 2d, $J=16.6$ Hz, 6.3 Hz; 16.6, 4.8 Hz, H-7a,7b), 1.37 (2×3H, 2d, $J=6.9$ Hz, H-16, H-17), 1.17 (3H, s, H-20), 1.13 (3H, s, H-18), 1.12 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.7, 142.5, 132.8, 131.6, 121.3, 114.2 (Ar), 69.1 (C-6), 57.3 (C-5), 43.0 (C-3), 40.0 (C-1), 38.7 (C-10), 37.5 (C-7), 35.2 (C-18), 34.0 (C-4), 27.4 (C-15), 23.6 (C-20), 21.3, 22.4 (C-19), 20.9, 20.8 (C-16, C-17), 19.2 (C-2); *m/z* 302 (M^+, 69%), 287 (100), 269 (40), 227 (43), 199 (32), 69 (15); HRMS *m/z* calcd for $C_{20}H_{30}O_2$ (M^+) 302.2246, found 302.2238.

Totara-8,11,13-triene-6β,13-diol (9). Lithium aluminum hydride (50 mg) was added portionwise to a stirred solution of 6-oxototarol (**14**) (see below) (100 mg, 0.30 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.05 mL), NaOH (15%, 0.05 mL), and water (0.15 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave the diol **9** as an oil (79 mg, 90%); ν_{max} 3401 (OH), 1502 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06, 6.58 (2H, 2d $J=8.6$ Hz, H-11, H-12), 4.88 (1H, s, OH), 4.75 (1H, d, $J=4.8$ Hz, H-6α), 3.27 (1H, sept, $J=7.1$ Hz, H-15), 3.08, 3.01 (2H, dd,d, $J=17.0$, 4.8; 17.7 Hz H-7α 7β), 1.60 (3H, s, H-20), 1.38 (2×3H, 2d, $J=7.1$ Hz, H-16, H-17), 1.31 (3H, s, H-19), 1.08 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 152.7, 142.5, 132.8, 131.6, 121.3, 114.2 (Ar), 69.1 (C-6), 57.3 (C-5), 43.0 (C-3), 40.0 (C-1), 38.7 (C-10), 37.5 (C-7), 35.2 (C-18), 34.0 (C-4), 27.4 (C-15), 23.6 (C-20), 21.3, 22.4 (C-19), 20.9, 20.8 (C-16, C-17), 19.2 (C-2); *m/z* 302 (M^+, 44%), 269 (100), 227 (21), 199 (17), 149 (20), 69 (18); HRMS *m/z* calcd for $C_{20}H_{30}O_2$ (M^+) 302.2246, found 302.2248.

13-Acetoxy-7-methoxytotara-6,8,11,13-tetraene. Trimethyl orthoformate (1 mL) was added dropwise to a stirred suspension of 13-acetoxytotara-8,11,13-triene-7-one (see above, 350 mg, 1 mmol) and Amberlyst® 15 (400 mg) in methanol (10 mL) and the resulting solution warmed to 50 °C. After 1 h the reaction mixture was cooled, filtered through Celite and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (99:1) as the eluent gave the title compound (270 mg, 76%); mp 103 °C; ν_{max} 1758 (CO), 1676 (C=C), 1206 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.02, 6.90 (2H, 2d $J=8.5$ Hz, H-11, H-12), 4.97 (1H, d, $J=3.7$ Hz, H-6), 3.72 (3H, s, OCH_3), 3.66 (1H, sept, $J=7.1$ Hz, H-15), 2.34 (3H, s, $COCH_3$), 1.35, 1.21 (2×3H, 2d, $J=7.1$ Hz, H-16, H-17), 1.08 (2×3H, 2s, H-20, H-18), 0.99 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 169.9 ($COCH_3$), 153.8 (C-7), 149.1, 148.4, 136.3, 131.8, 123.1, 120.7 (Ar), 99.0 (C-6), 54.5 (OCH_3), 49.5 (C-5), 41.2 (C-3), 39.8 (C-10), 36.8 (C-1), 33.3 (C-4), 32.9 (C-18), 29.8 (C-15), 22.0 (C-20), 21.9 (C-19), 21.7, 21.6 (C-16, C-17), 20.0 ($CO\mathit{C}H_3$), 19.2 (C-2); *m/z* 356 (M^+, 49%), 342 (50), 300 (100), 285 (37), 267 (58), 243 (18), 215 (33), 128 (10), 83 (12), 55 (28); HRMS *m/z* calcd for $C_{23}H_{32}O_3$ (M^+) 356.2352, found 356.2364.

7-Methoxytotara-6,8,11,13-tetraen-13-ol (10). Lithium aluminum hydride (100 mg) was added portionwise to a stirred solution of 13-acetoxy-7-methoxytotara-6,8,11,13-tetraene (300 mg, 0.8 mmol) in THF (10 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.1 mL), NaOH (15%, 0.1 mL), and water (0.3 mL), filtered and

the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave the methoxytotarol **10** (190 mg, 72%); mp 238 °C; ν_{max} 3218 (OH), 1646 (C=C), 1570, 1486, 1342, 1188 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.86, 6.60 (2H, 2d, J = 8.4 Hz, H-11, H-12), 4.95 (1H, d, J = 3.7 Hz, H-6), 4.80 (1H, s, OH), 3.72 (3H, s, OCH_3), 3.72 (1H, sept, J = 7.0 Hz, H-15), 1.44, 1.32 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.06 (3H, s, H-20), 1.05 (3H, s, H-18), 0.98 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 154.1 (C-7), 153.4, 144.3, 131.4, 130.9, 120.5, 116.0 (Ar), 98.8 (C-6), 54.4 (OCH_3), 49.8 (C-5), 41.2 (C-3), 39.4 (C-10), 36.9 (C-1), 33.1 (C-4), 32.8 (C-18), 29.8 (C-15), 21.9 (C-20), 21.3, 21.1 (C-16, C-17), 20.2 (C-19), 19.2 (C-2); m/z 314 (M^+, 17%), 300 (100), 285 (41), 267 (53), 243 (18), 215 (52), 181 (19), 131 (23), 83 (16), 69 (45), 55 (32); HRMS m/z calcd for $C_{21}H_{30}O_2$ (M^+) 314.2246, found 314.2249.

13-Hydroxytotara-8,11,13-trien-7-one (13).[21] Sodium hydride (10 mg) was added to a stirred solution of 13-acetoxytotara-8,11,13-trien-7-one (see above, 72 mg, 0.2 mmol) in methanol (10 mL) at room temperature under argon. The reaction mixture was stirred for 14 h and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave ketone **13** (50 mg, 83%); mp 240 °C; lit.[21] 240–241 °C; ^1H NMR ($CDCl_3$) δ 6.81, 6.64 (2H, 2d, J = 8.5 Hz, H-11, H-12), 3.40 (1H, sept, J = 6.9 Hz, H-15), 2.46 (2H, m, H-6a,b), 1.17, 1.08 (2×3H, 2d, J = 6.9 Hz, H-16, H-17), 0.87 (3H, s, H-20), 0.83 (3H, s, H-18), 0.72 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 205.9 (C-7), 156.5, 149.2, 145.8, 145.0, 122.2, 121.2 (Ar), 49.9 (C-5), 42.8 (C-3), 40.0 (C-1), 39.8 (C-6), 39.0 (C-10), 34.2 (C-4), 32.8 (C-18), 29.8 (C-15), 23.5 (C-20), 21.7 (C-9), 21.4, 20.9 (C-16, C-17), 20.1 (C-2).

13-Methoxytotara-6,8,11,13-tetraene.[6] Dilute hydrochloric acid (10%, 1 mL) was added dropwise to a stirred solution of 7α-acetoxy-13-methoxytotara-8,11,13-triene[6] (350 mg, 0.98 mmol) in methanol (20 mL) and the reaction heated at reflux for 1.5 h. The volatiles were removed under reduced pressure and the residue resuspended in ethyl acetate (100 mL), washed with brine (3×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (99:1) as the eluent gave the title tetraene (161 mg, 55%); mp 84 °C; ^1H NMR ($CDCl_3$) δ 6.97, 6.71 (2H, 2d, J = 8.5 Hz, H-11, H-12), 6.89 (1H, dd, J = 10.1, 3.1 Hz, H-7), 6.03 (1H, dd, J = 10.1, 3.1 Hz, H-6), 3.77, (3H, s, OCH_3), 3.49 (1H, sept, J = 7.0 Hz, H-15), 1.33, 1.33 (2×3H, d, J = 7.0 Hz, H-16, H-17), 1.04 (3H, s, H-20), 1.01 (3H, s, H-18), 0.96 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 156.5, 141.7, 131.8, 131.3 (Ar), 130.5 (C-6), 124.4 (C-7), 120.0, 110.1 (Ar), 55.2 (OCH_3), 50.0 (C-5), 41.0 (C-3), 38.2 (C-10), 36.6 (C-1), 32.5 (C-4), 32.5 (C-18), 27.3 (C-15), 24.5 (C-20), 22.3 (C-19), 21.3, 21.0 (C-16, C-17), 19.1 (C-2).

13-Methoxytotara-8,11,13-trien-6-one. *m*-Chloroperoxybenzoic acid (85%, 4.0 g) was added to a stirred solution of 13-methoxytotara-6,8,11,13-tetraene (3.8 g, 12.7 mmol) in CH_2Cl_2 (40 mL) at room temperature under argon. The solution was stirred for 2 h, diluted with CH_2Cl_2 (100 mL), washed with aqueous solutions of KI (10%, 2×20 mL), $Na_2S_2O_3$ (10%, 2×20 mL), $NaHCO_3$ (2×20 mL), and brine (2×20 mL), dried ($MgSO_4$) and concentrated in vacuo to give the 6,7-α-epoxide. *p*-Toluenesulfonic acid (0.5 g) was added to a stirred solution of the crude epoxide (4.0 g) in benzene (50 mL) and the solution was heated under reflux for 1 h before filtration through solid $NaHCO_3$ and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave the title 6-one (3.2 g, 84%); mp 96–98 °C; ν_{max} 1709 (C=O), 1630 (C=C); ^1H NMR ($CDCl_3$) δ 7.60, 7.15 (2H, 2d, J = 8.8 Hz, H-11, H-12), 3.80 (3H, s, OCH_3), 3.60, 3.50 (2H, 2d, J = 21.1z H, H-7a,b), 3.10 (1H, br s, H-15), 1.33, 1.33 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.30 (3H, s, H-20), 1.12 (3H, s, H-18), 1.07 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 209.7 (C-6), 141.5, 133.2, 130.8, 122.7, 122.0, 110.2 (Ar), 55.3 (OCH_3), 61.9 (C-5), 44.0 (C-7), 42.8 (C-3), 40.5 (C-10), 39.5 (C-1), 32.9 (C-18), 32.5 (C-4), 28.2 (C-15), 25.3 (C-20), 21.8 (C-19), 20.5, 20.4 (C-16, C-17), 19.0 (C-2); m/z 314 (M^+, 100%), 299 (92), 281 (20), 257 (32), 229 (52), 217 (23), 69 (30), 55 (18), 41 (28); HRMS m/z calcd for $C_{21}H_{30}O_2$ (M^+) 314.2246, found 314.2245.

6-Oxototara-8,11,13-trien-13-ol (14). Boron tribromide (1.0 M, 1 mL) was added dropwise to a stirred solution of 13-methoxytotara-8,11,13-trien-6-one (45 mg, 0.14 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 aqueous solutions of $Na_2S_2O_3$ (10%, 3×25 mL), $NaHCO_3$ (3×25 mL), brine (3×25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (12:1) as the eluent gave the hydroxy ketone **14** (39 mg, 91%); mp 70 °C; ν_{max} 3454 (OH), 1696 (C=O), 1632 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.04, 6.63 (2H, 2d, J = 8.5 Hz, H-11, H-12), 5.10 (1H, s, OH), 3.67, 3.49 (2H, 2d, J = 21.2 Hz, H-7a,b), 3.11 (1H, br s, H-15), 1.35, 1.33 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.33 (3H, s, H-20), 1.11 (3H, s, H-18), 1.08 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 210.1 (C-6), 153.0, 141.6, 131.1, 122.5, 115.2 (Ar), 62.0 (C-5), 43.9 (C-7), 42.7 (C-3), 40.5 (C-10), 39.3 (C-1), 32.8 (C-18), 32.4 (C-4), 27.9 (C-15), 25.2 (C-20), 21.7 (C-19), 20.4, 20.2 (C-16, C-17), 18.9 (C-2); m/z 300 (M^+, 78%), 285 (100), 267 (21), 243 (31), 215 (52), 173 (15), 69 (28); HRMS m/z calcd for $C_{20}H_{28}O_2$ (M^+) 300.2089, found 300.2091.

13-Acetoxy-6α-bromototara-8,11,13-trien-7-one.[7,22] A solution of bromine (250 mg, 2 mmol) in glacial acetic acid (5 mL) was added dropwise over a period of 10 min to a stirred solution of 13-acetoxytotara-8,11,13-trien-7-one (500 mg, 2 mmol) in glacial acetic acid (20 mL) containing 1 drop of HBr. After 30 min the mixture was concentrated in vacuo, and the resultant residue purified by column chromatography on silica gel using petrol:ethyl acetate (12:1) as the eluent gave the title bromoketone (454 mg, 74%); mp 174–175 °C, lit. 176–177 °C. ^1H NMR ($CDCl_3$) δ 7.14, 7.13 (2H, 2d, J = 8.7 Hz, H-11, H-12), 4.56 (1H, d, J = 7.5 Hz, H-6), 3.47 (1H, sept, J = 7.0 Hz, H-15), 2.36 (3H, s, $COCH_3$), 2.16 (1H, d, J = 7.5 Hz, H-5), 1.40, 1.26 (2×3H, 2d, J = 7.0 Hz, H-16,

H-17), 1.22 (3H, s, H-20), 1.15 (3H, s, H-18), 1.09 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 193.6 (C-7), 169.2 (*C*OCH_3), 149.6, 148.2, 139.2, 133.1, 127.0, 120.1 (Ar), 56.6 (C-6), 51.5 (C-5), 41.7 (C-3) 38.6 (C-10), 37.6 (C-1), 34.9 (C-4), 34.2 (C-18), 28.1 (C-15), 23.8 (C-20), 21.8 (C-19), 21.3, 21.2 (C-16, C-17), 18.3 (C-2).

6α-Bromo-13-hydroxytotara-8,11,13-trien-7-one (15).[7,22] Sodium hydride (catalytic) was added to a stirred solution of 13-acetoxy-6-α-bromototara-8,11,13-trien-7-one (300 mg, 0.7 mmol) in methanol (10.0 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave **15** as a solid (223 mg, 88%); mp 219 °C, lit.[7] 218–220 °C. ^{1}H NMR ($CDCl_3$) δ 6.98, 6.82 (2H, J = 8.4 Hz, H-11, H-12), 4.86 (1H, br s, OH), 4.55 (1H, d, J = 7.5 Hz, H-6), 3.50 (1H, sept, J = 7.1 Hz, H-15), 2.15 (1H, d, J = 7.5 Hz, H-5), 1.49, 1.37, (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 1.22 (3H, s, H-20), 1.14 (3H, s, H-18), 1.06 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 194.3 (C-7), 153.6, 144.9, 133.9, 131.3, 120.3, 120.0 (Ar), 57.2 (C-6), 52.2 (C-5), 41.9 (C-3), 38.5 (C-10), 37.9 (C-1), 35.2 (C-4), 34.3 (C-18), 28.1 (C-15), 24.1 (C-20), 21.9, 21.9 (C-16, C-17), 20.8 (C-19), 18.3 (C-2).

13-Acetoxytotara-5,8,11,13-tetraen-7-one.[7,22] 13-Acetoxytotara-6α-bromotetraen-8,11,13-trien-7-one (see above, 260 mg, 0.6 mmol), LiBr (520 mg), and $NaHCO_3$ (120 mg) were suspended in DMF (5 mL) with stirring and the mixture was heated at 100 °C for 48 h. The reaction mixture was diluted with brine (100 mL), 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. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave the conjugated enone (190 mg, 91%); mp 162–163 °C, lit.[22] 164–165 °C; ^{1}H NMR ($CDCl_3$) 7.38, 7.15 (2H, J 8.8 Hz, H-11, H-12), 6.42 (1H, s, H-6), 4.40 (1H, sept, J = 7.1 Hz, H-15), 2.35 (3H, s, $COCH_3$), 1.51 (3H, s, H-18), 1.39, 1.23 (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 1.34 (3H, s, H-19), 1.25 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) δ 188.5 (C-7), 170.1 (C-5), 169.5 (*C*OCH_3), 151.9, 148.3, 140.0, 131.4, 127.0 (Ar), 126.3 (C-6), 123.1 (Ar), 41.6 (C-10), 39.8 (C-3), 38.0 (C-1), 36.7 (C-4), 33.2 (C-18), 31.9 (C-20), 29.4 (C-19), 26.8 (C-15), 21.4, 21.3 (C-16, C-17), 21.2 (CO*C*H_3), 18.6 (C-2).

13-Hydroxytotara-5,8,11,13-tetraen-7-one (16).[22] Sodium hydride (catalytic) was added to a stirred solution of 13-acetoxytotara-5,8,11,13-tetraen-7-one (70 mg, 0.2 mmol) in methanol (5 mL) at room temperature under argon and the reaction mixture was stirred for 14 h and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave the hydroxy compound **16** (53 mg, 89%); mp 243 °C, lit.[22] 243–244.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.23, 6.95 (2H, J = 8.8 Hz, H-11, H-12), 6.43 (1H, s, H-6), 5.75 (1H, s, OH), 4.44 (1H, sept J = 7.1 Hz, H-15), 1.49, 1.36 (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 1.47 (3H, s, H-18), 1.32 (3H, s, H-19), 1.24 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) 189.9 (C-7), 170.8 (C-5), 154.0, 147.2, 134.1, 131.2, (Ar), 126.5 (C-6), 123.4 120.8 (Ar), 41.5 (C-10), 40.1 (C-3), 38.4 (C-1), 36.9 (C-4), 33.5 (C-18), 32.1 (C-20), 29.5 (C-19), 27.1 (C-15), 20.7, 21.1 (C-16, C-17), 18.8 (C-2).

13-Acetoxy-6α,7α-epoxytotara-8,11,13-triene (17).[11] *m*-Chloroperoxybenzoic acid (85%, 4.0 g) was added to a stirred solution of 13-acetoxytotara-6,8,11,13-tetraene (**11**) (2.6 g, 8 mmol) in CH_2Cl_2 (50 mL) at room temperature under argon. The solution was stirred for 2 h, diluted with CH_2Cl_2 (100 mL), washed with aqueous solutions of KI (10% w/w, 2×20 mL), $Na_2S_2O_3$ (10%, 2×20 mL), $NaHCO_3$ (2×20 mL), and brine (2×20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave epoxide **17** (2.5 g, 92%); mp 137–139 °C, lit.[11] 138–139 °C; ^{1}H NMR ($CDCl_3$) δ 6.97, 6.86 (2H, J = 8.6 Hz, H-11, H-12), 3.83 (1H, d, J = 4.6 Hz, H-7β), 3.48 (2H, m, H-6, H-15), 2.25 (3H, s, $COCH_3$), 1.29, 1.27 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.16 (3H, s, H-20), 1.09 (3H, s, H-18), 1.03 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 169.6 (*C*OCH_3), 149.9, 147.2, 140.0, 131.0, 123.9, 121.6 (Ar), 54.1 (C-7), 52.9 (C-6), 47.3 (C-5), 40.8 (C-3), 38.8 (C-10), 37.0 (C-1), 33.2 (C-4), 32.5 (C-18), 28.7 (C-15), 25.4 (C-20), 22.1 (C-19), 21.4, 21.4 (C-16, C-17), 21.3 (CO*C*H_3), 18.8 (C-2).

6α,7α-Epoxytotara-8,11,13-trien-13-ol (18). Sodium hydride (catalytic) was added to a stirred solution of the epoxyacetate **17** (100 mg, 0.3 mmol) in methanol (10 mL) at room temperature under argon. The mixture was stirred for 14 h and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (12:1) as the eluent gave the epoxyalcohol **18** (74 mg, 82%); mp 175 °C; ν_{max} 3230 (OH), 1583 (C=C), 1202 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.90, 6.65 (2H, J = 8.4 Hz, H-11, H-12), 4.90 (1H, s, OH), 3.95, 3.59 (2H, 2d, J = 4.7 Hz, H-6, H-7), 3.62 (1H, sept, J = 7.1 Hz, H-15), 1.46 (2×3H, 2t, J = 7.1 Hz, H-16, H-17), 1.23 (3H, s, H-20), 1.18 (3H, s, H-18), 1.12 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.3, 144.6, 134.8, 130.4, 121.3, 116.7 (Ar), 54.2 (C-7), 53.1 (C-6), 47.7 (C-5), 40.8 (C-3), 38.3 (C-10), 37.1 (C-1), 33.1 (C-4), 32.4 (C-18), 28.8 (C-15), 25.5 (C-20), 22.0 (C-19), 20.8, 20.7 (C-16, C-17), 18.8 (C-2); m/z 300 (M^+, 77%), 285 (100), 243 (29), 215 (73), 203 (42), 69 (51), 55 (22), 41 (41); anal. calcd for $C_{20}H_{28}O_2$: C, 79.9; H, 9.3; found: C,79.7; H, 9.3.

13-Acetoxy-6α,18-epoxytotara-8,11,13-triene.[11] Iodine (0.6 g, 2.4 mmol) was added to a stirred solution of 13-acetoxytotara-8,11,13-trien-6α-ol (0.5 g, 1.5 mmol) and lead tetraacetate (1.1 g, 2.5 mmol) in benzene at room temperature under argon. The mixture was stirred for 2 h, diluted with benzene (150 mL), and the solution was washed with aqueous $Na_2S_2O_3$ (10% w/w, 2×50 mL), $NaHCO_3$ (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 the title tetrahydrofuranyl compound (0.35 g, 70%); mp 148–149 °C, lit.[11] 146–148 °C. ^{1}H NMR ($CDCl_3$) δ 7.11, 6.84 (2H, 2d, J = 8.6 Hz, H-11, H-12), 4.10 (1H, m, H-6β), 3.74, 3.50 (2H, 2d, J = 7.1 Hz, H-18a,b), 3.46 (1H, m, H-7α), 3.30 (1H, sept, J = 7.1 Hz, H-15), 2.66 (1H, dd, J = 15.9, 8.4 Hz, H-7β), 2.31 (3H, s, $COCH_3$), 1.26 (2×3H, 2t, J = 7.3 Hz, H-16, H-17), 1.19 (2×3H, 2s, H-20,

H-19); ^{13}C NMR ($CDCl_3$) δ 169.9 ($COCH_3$), 147.7, 146.2, 137.8, 133.2, 123.9, 121.8 (Ar), 83.8 (C-18), 71.4 (C-6), 56.3 (C-5), 40.0 (C-10), 39.3 (C-7), 37.5 (C-4), 36.7 (C-1), 35.4 (C-3), 27.6 (C-15), 23.8 (C-20), 21.6 ($COCH_3$), 21.0, 21.0 (C-16, C-17), 20.4 (C-2), 19.1 (C-19).

6α,18-Epoxytotara-8,11,13-trien-13-ol (19). Lithium aluminium hydride (100 mg) was added portionwise to a stirred solution of 13-acetoxy-6α,18-epoxytotara-8,11,13-triene (see above, 200 mg, 0.6 mmol) in THF (20 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.1 mL), NaOH (15%, 0.1 mL), and water (0.3 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (9:1) as the eluent gave the alcohol **19** (170 mg, 96%); mp 209 °C; ν_{max} 3300 (OH), 1192, 1077 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.96, 6.61 (2H, 2d, J = 8.4 Hz, H-11, H-12), 5.50 (1H, br s, OH), 4.14 (1H, q, J = 7.1 Hz, H-6β), 3.76, 3.52 (2H, 2d, J = 7.1 Hz, H-18), 3.64 (1H, m, H-7α), 3.32 (1H, m, H-15), 2.68 (1H, dd, J = 15.9, 8.4 Hz, H-7β), 1.38, 1.36 (2×3H, 2t, J = 7.0 Hz, H-16, H-17), 1.21 (3H, s, H-20), 1.19 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 152.9, 140.7, 132.5, 132.2, 123.4, 114.8 (Ar), 83.5 (C-18), 71.6 (C-6), 56.2 (C-5), 39.7 (C-10), 39.3 (C-7), 36.8 (C-4), 36.4 (C-1), 35.1 (C-3), 27.5 (C-15), 23.7 (C-20), 20.1 (C-19), 20.0, 20.0 (C-16, C-17), 18.2 (C-2); m/z 300 (M^+, 63%), 285 (35), 255 (23), 216 (100), 201 (25), 157 (16), 95 (17); anal. calcd for $C_{20}H_{28}O_2$: C, 79.9; H, 9.4; found: C, 79.7; H, 9.6.

13-Methoxytotara-8,11,13-trien-6β-ol. Lithium aluminum hydride (400 mg) was added portionwise to a stirred solution of 13-methoxytotara-8,11,13-trien-6-one (see above) (2.7 g, 8.6 mmol) in THF (50 mL) under argon at room temperature. The reaction mixture was stirred for 2 h, quenched with water (0.4 mL), NaOH (15%, 0.4 mL), and water (1.2 mL), filtered and the filtrate was concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave the title alcohol (2.15 g, 79%); mp 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 7.18, 6.81 (2H, 2d, J = 8.8 Hz, H-11, H-12), 4.75 (1H, br s, H-6α), 3.81 (3H, s, OCH_3), 3.26 (1H, m, H-15), 3.11 (1H, dd, J = 17.2, 5.1 Hz, H-7α), 3.00 (1H, d, J = 17.2 Hz, H-7β), 1.62 (3H, s, H-20), 1.36, 1.33 (2 3H, 2d, J = 7.1 Hz, H-16, H-17), 1.32 (3H, s, H-19), 1.08 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 156.3, 141.4, 133.4, 129.7, 123.5, 110.0 (Ar), 65.8 (C-6), 54.9 (OCH_3), 52.2 (C-5), 43.0 (C-3), 42.9 (C-1), 39.4 (C-7), 37.1 (C-10), 33.9 (C-4), 33.4 (C-18), 27.4 (C-15), 27.2 (C-20), 23.6 (C-19), 20.3, 20.2 (C-16, C-17), 19.6 (C-2); m/z 316 (M^+, 69%), 298 (100), 241 (24), 213 (17), 171 (11), 69 (18); anal. calcd for $C_{21}H_{32}O_2$: C, 79.7; H, 10.4; found: C, 79.9; H, 10.4.

6β,20-Epoxy-13-methoxytotara-8,11,13-triene (20). Iodine (1.2 g, 4.7 mmol) was added to a stirred solution of 13-methoxytotara-8,11,13-trien-6β-ol (1 g, 3.2 mmol) and lead tetraacetate (2.2 g, 4.8 mmol) in benzene at room temperature under argon. The reaction mixture was stirred for 2 h, diluted with benzene (150 mL), washed with aqueous $Na_2S_2O_3$ (10% w/w, 2×50 mL), $NaHCO_3$ (2×50 mL), brine (2×50 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (19:1) as the eluent gave the epoxyether **20** (0.90 g, 90%); mp 155 °C; ν_{max} 1642 (C=C), 1200, 1180 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.03, 6.76 (2H, 2d, J = 8.6 Hz, H-11, H-12), 4.57 (1H, br s, H-6α), 4.01, 3.73 (2H, 2d, J = 7.2 Hz, H-20a, H-20b), 3.83 (3H, s, OCH_3), 3.22 (1H, m, H-15), 3.13, 2.99 (2H, 2d, J = 17.2 Hz, H-7α,β), 1.37, 1.33 (2×3H, 2d, J = 7.0 Hz, H-16, H-17), 1.11 (3H, s, H-19), 1.02 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 157.4, 142.7, 135.0, 132.2, 120.5, 109.3 (Ar), 78.4 (C-20), 77.6 (C-6), 55.2 (OCH_3), 54.6 (C-5), 45.0 (C-10), 40.0 (C-1), 40.0 (C-3), 33.6 (C-18), 31.3 (C-4), 28.3 (C-15), 28.2 (C-7), 22.7 (C-19), 20.5, 20.4 (C-16, C-17), 18.9 (C-2); m/z 314 (M^+, 96%), 269 (100), 241 (11), 185 (18); HRMS calcd for $C_{21}H_{30}O_2$ (M^+) 314.2246, found 314.2243.

Attempted demethylation of 6β,20-epoxy-13-methoxytotara-8,11,13-triene (20). Boron tribromide (10 mL, 1.0 M) was added dropwise to a stirred solution of compound **20** (600 mg, 1.9 mmol) in CH_2Cl_2 (20 mL) at room temperature under argon. The reaction mixture was stirred for 30 min, diluted with CH_2Cl_2 (250 mL), washed with aqueous $Na_2S_2O_3$ (10% w/w, 3×75 mL), $NaHCO_3$ (3×75 mL), brine (3×75 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography on silica gel using petrol:ethyl acetate (12:1) as the eluent gave in order of elution: 13,20-dihydroxytotara-6,8,11,13-tetraene (**21**) as an oil (222 mg, 39%); ν_{max} 3415 (OH), 1661 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.85, 6.47 (2H, 2d, J = 8.3 Hz, H-11, H-12), 6.89 (1H, dd, J = 10.1, 3.1 Hz, H-7), 5.96 (1H, dd, J = 10.1, 3.1 Hz, H-6), 5.06 (2H, s, OH), 3.62, 3.38 (2H, 2t, J = 6.4 Hz, H-20α,β), 3.38 (1H, sept, J = 7.1 Hz, H-15), 1.30, 1.28 (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 0.92 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 153.4, 144.6, 132.4, 130.1 (C-6), 124.3 (C-7), 123.9, 114.2 (Ar), 59.8 (C-20), 50.0 (C-5), 43.4 (C-10), 40.7 (C-3), 32.4 (C-4), 32.3 (C-18), 30.7 (C-1), 27.2 (C-15), 22.9 (C-19), 21.0, 20.8 (C-16, C-17), 18.6 (C-2); m/z 300 (M^+, 32%), 270 (100), 199 (21), 55 (10); HRMS, calcd for $C_{20}H_{28}O_2$ (M^+) 300.2089, found 300.2093; 6β,13,20-trihydroxytotara-8,11,13-8,11,13-triene (**22**) as an oil (265 mg, 44%); ν_{max} 3684, 3615, 3453 (OH), 1273 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.76, 6.55 (2H, 2d J = 8.1 Hz, H-11, H-12), 5.29 (1H, d, J = 2.1 Hz, H-6α), 4.27 (1H, d, J = 8.2 Hz, H-20a), 3.37 (1H, sept, J = 7.1 Hz, H-15), 2.80 (1H, dd, J = 8.2, 1.6 Hz, H-20b), 1.28, 1.27 (2×3H, 2d, J = 7.1 Hz, H-16, H-17), 1.09 (3H, s, H-19), 0.76 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 152.5, 139.2, 138.3, 128.4, 117.4, 114.1 (Ar),67.6 (C-20), 66.9 (C-6), 42.8 (C-5), 41.2 (C-3), 37.0 (C-10), 33.6 (C-4), 32.7 (C-18), 29.4 (C-1), 29.2 (C-7), 26.5 (C-15), 21.8, 21.6 (C-16, C-17), 20.8 (C-19), 18.9 (C-2); m/z 300 (M^+-H_2O, 22%), 270 (100), 255 (47), 202 (22), 199 (21), 171 (24), 157 (20), 145 (18), 41 (12); HRMS calcd for $C_{20}H_{28}O_2$ 300.2089, found 300.2089.

Biology

Minimum inhibitory concentrations (MIC) were determined by the double agar dilution method with Mueller–Hinton agar as described previously.[3] Liver mitochondria were prepared from female Wistar rats

(200–300 g) by homogenization followed by differential centrifugation at 4 °C in an isolation medium containing 250 mM sucrose, 5 mM Tris–HCl and 1 mM EGTA, pH 7.4, and stored on ice at about 50 mg protein/mL.[23] Protein concentrations were determined by the biuret assay[24] using bovine serum albumin as a standard. To measure respiration rate mitochondria (1 mg protein/mL) were incubated in medium containing 120 mM KCl, 5 mM KH_2PO_4, 2 mM $MgCl_2$, 10 mM Hepes–KOH, 1 mM EGTA, pH 7.2, at 25 °C in an oxygen electrode chamber thermostatted at 25 °C (Rank Brothers, Bottisham, Cambridge, UK). The respiratory substrates used were either glutamate and malate (5 mM of each), succinate (5 mM) or ascorbate (5 mM) and TMPD (500 μM). When succinate or ascorbate/TMPD were used as respiratory substrates rotenone (13 μM) was also present. Mitochondria were preincubated with respiratory substrates for up to 1 min, then totarol (**1**), 7-oxototarol (**13**) or an equivalent volume of ethanol carrier was added and the rate of coupled respiration was measured; ADP (200 μM) was then added and the rate of phosphorylating respiration measured; finally, the uncoupler FCCP (333 nM) was added and the rate of uncoupled respiration measured. The oxygen electrode was calibrated assuming an oxygen concentration of 475 nmol O atoms/mL in air-equilibrated medium at 25 °C.[25] Respiration rates in the presence of various concentrations of totarol (**1**) or 7-oxototarol (**13**) were determined in duplicate and the effects of them on coupled, phosphorylating or uncoupled respiration for the three different respiratory substrates are expressed as a percentage of the control incubations in the presence of ethanol carrier. Data are the means of determinations on at least three separate mitochondrial preparations ±S.E.M.

X-ray single crystal analysis

Crystallographic data for 7-oxototarol (13). $C_{20}H_{28}O_2$, orthorhombic, space group $P2_12_12_1(19)$,[26] $a = 10.400(1)$, $b = 12.002(1)$, $c = 13.852(1)$ Å, $V = 1729.0(3)$ Å^3, $Z = 4$, $D_C = 1.154$ Mg m^{-3}, $T = 173(2)$ K, Mo K_α radiation ($\lambda = 0.71073$ Å), $\mu = 0.72$ cm^{-1}. A Siemens P4 diffractometer was used to measure 2985 independent reflections ($4° \leq 2\theta \leq 52°$) of which 1925 "observed" had $I_{net} > 2\,\sigma\,(I_{net})$. No absorption correction was made and the structure was solved by direct methods[27] and refined on F_o^2 using all data to give R ("observed") 0.057, wR_2 (all data, on F_o^2) 0.104.[28] The absolute configuration was not determined and all hydrogen atoms were located by difference Fourier maps and refined with individual isotropic thermal parameters. The final maximum shift/error was 0.006 with $\Delta\rho$ excursions −0.20 to 0.17 e Å^{-3}.

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