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Triumphalone, a diketone from the volatile oil of the leaves of *Melaleuca triumphalis*, and its spontaneous conversion into isotriumphalone

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Abstract

The major component (35–65%) of the volatile oil obtained by steam distillation of the leaves of *Melaleuca triumphalis* has been identified as (*rel*)-1β-pentyl-1α,6α-dihydroxy-3,3,5,5-tetramethylcyclohexa-2,4-dione (trivial name triumphalone). Relative stereochemistry was established by nuclear Overhauser experiments and X-ray studies on the 2-(3,5-dinitrobenzoic acid) derivative. The remainder of the oil was composed of mono- and sesquiterpene hydrocarbons and alcohols. On prolonged standing the presence of a rearrangement product of triumphalone was observed which was characterized as (*rel*)-1β-pentyl-1α,3α-dihydroxy-4,4,6,6-tetramethylcyclohexa-2,5-dione (trivial name isotriumphalone), presumably arising from an acid catalyzed shift of the pentyl group from C-1 to C-2.

Keywords: *Melaleuca triumphalis*; Myrtaceae; Leaf oil; Triumphalone; (*rel*)-1β-Pentyl-1α,6α-dihydroxy-3,3,5,5-tetramethylcyclohexa-2,4-dione; Isotriumphalone; (*rel*)-1β-Pentyl-1α,3α-dihydroxy-4,4,6,6-tetramethylcyclohexa-2,5-dione

1. Introduction

Melaleuca triumphalis Craven is a newly discovered broad-leafed species that was described in 1998. It is a shrub, growing up to 2.5 m in height, and is known only from the Victoria River Gorge and associated gorges in the Northern Territory, Australia (Craven, 1998). It grows in sites with perennial seepage near the base of ephemeral waterfalls, either at the top of scree slopes or in crevices near the base of the cliff (Craven, 1998). It would appear to be most closely related to *M. nervosa* (Lindl.) Cheel and *M. fluviatilis* Barlow. The leaf oils from these two species, obtained in a yield of less than 0.3% in each case, contain a range of sesquiterpenes, both hydrocarbon and oxygenated, in the case of *M. nervosa* and a monoterpenic hydrocarbon oil, in the case of *M. fluviatilis* (Brophy and Doran, 1996).

Steam distillation of the leaves of *M. triumphalis* yielded an oil (as 1–1.5% v/w based on fresh leaves) which contained a mixture of mono- and sesquiterpene hydrocarbons and alcohols that totalled approximately 30–40% of the oil. The remainder was made up of a new non-terpenoid hydroxy ketone. This communication relates to the structural identification of this compound and its ready rearrangement.

2. Results and discussion

The steam volatile oil of *M. triumphalis* contained as its major components α-pinene (16–30%), β-caryophyllene (2–5%), caryophyllene oxide (4–8%), spathulenol (1–3%) and triumphalone (35–65%). Chromatography on SiO_2

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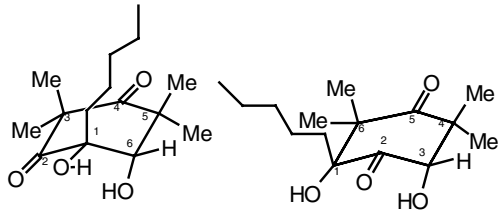
and elution with pentane separated out the hydrocarbons, while elution with 5–15% CH_2Cl_2 in pentane eluted the oxygenated terpenoids. Elution with 30–80% CH_2Cl_2 in pentane eluted substantially pure triumphalone as a colourless oil.



Triumphalone (**1**) gave a MH^+ ion at *m/z* 271.1906 (FAB), consistent with a protonated molecular ion, $C_{15}H_{27}O_4$ (271.1917). The IR spectrum (neat) revealed absorption bands at 3484, 1728 and 1698 cm^{-1}, consistent with the presence of hydroxy and ketone carbonyl groups in the molecule. The EI mass spectrum revealed ions at *m/z* 270 (M^+ 0.5%), 236 (5), 221 (1), 193 (5), 171 (18), 166 (10), 152 (8), 137 (15), 109 (25), 101 (75), 83 (25), 71 (60), 55 (30), 43 (100).

The ^{13}C NMR spectrum of triumphalone (Table 1) revealed resonances for 15 carbons, including five methyls, four methylenes, an oxymethine and five quaternary carbons made up of two carbonyls and three sp3 carbons, one of which was oxygen-bearing. The ^1H spectrum (Table 1) indicated an pentyl spin system accounting for the four methylenes and one methyl. Resonances for the remaining methyls and the oxymethine were all observed as singlets. Direct C–H (HSQC) and long-range C–H (HMBC) correlation spectra were used to establish the structure of **1** revealing a cyclohexane ring system substituted with two pairs of geminal dimethyls (C-3 and C-5), two carbonyls (C-2 and C-4), an oxymethine (C-6) and a quaternary carbon substituted with a hydroxyl and an pentyl group.

Table 1
NMR spectral data for triumphalone (**1**) and isotriumphalone (**2**), run in $CDCl_3$

C/H	**1**		**2**	
	^1H	^{13}C	^1H	^{13}C
1		82.2		87.7
2		214.4		213.2
3		55.2	4.78 *s*	76.6
4		215.7		46.9
5		48.3		221.7
6	3.78 *s*	77.3		53.6
3-Me_2	1.37 *s*	25.5		
	1.27 *s*	23.3		
4-Me_2			1.23 *s*	26.9
			1.09 *s*	20.1
5-Me_2	1.35 *s*	27.3		
	1.28 *s*	25.5		
6-Me_2			1.18 *s*	17.6
			0.92 *s*	22.8
1′-CH2	1.63 *dt* (12.8, 3.5)	35.1	2.81 *dt* (17.5, 8.1)	39.7
	1.40 *m*		2.56 *dt* (17.5, 8.1)	
2′-CH2	1.63 *m*	22.9	1.63 *m*	23.3
3′-CH2	1.30 *m*	31.9	1.30 *m*	31.6
	1.24 *m*		1.24 *m*	
4′-CH2	1.30 *m*	22.7	1.30 *m*	22.6
	1.24 *m*		1.24 *m*	
5′-Me	0.85 *t* (7.3)	14.1	0.90 *t* (7.3)	14.1

The relative stereochemistry in **1** was examined by means of a series of 1D nuclear Overhauser (nOe) experiments. It was found that irradiation of the methyls at δ 1.35 (at C-5) and 1.37 (at C-3) led to the enhancement of the C-1′ methylene proton at δ 1.63 and that the oxymethine proton at H-6 also interacted with the same C-1′ methylene proton and the methyl resonating at δ 1.35. These findings suggested that **1** existed in a half-chair conformation with the two hydroxy groups on the same face of the molecule.

In order to confirm the relative stereochemistry of **1** it was converted into the corresponding 6-(3,5-dinitrobenzoyl) ester, recrystallized (m.p. 109–110 °C), and an X-ray crystal structure determined (Fig. 1). This confirmed the half-chair conformation of the cyclohexane ring and established the absolute structure of **1** as (*rel*)-1(*S*),6(*S*)-1β-pentyl-1α,6α-dihydroxy-3,3-5,5-tetramethylcyclohexa-2,4-dione.

During the course of the isolation and structural elucidation work on triumphalone it became apparent that additional resonances were appearing in the NMR spectrum. The first isolation of triumphalone from the crude oil and initial NMR data revealed triumphalone as the

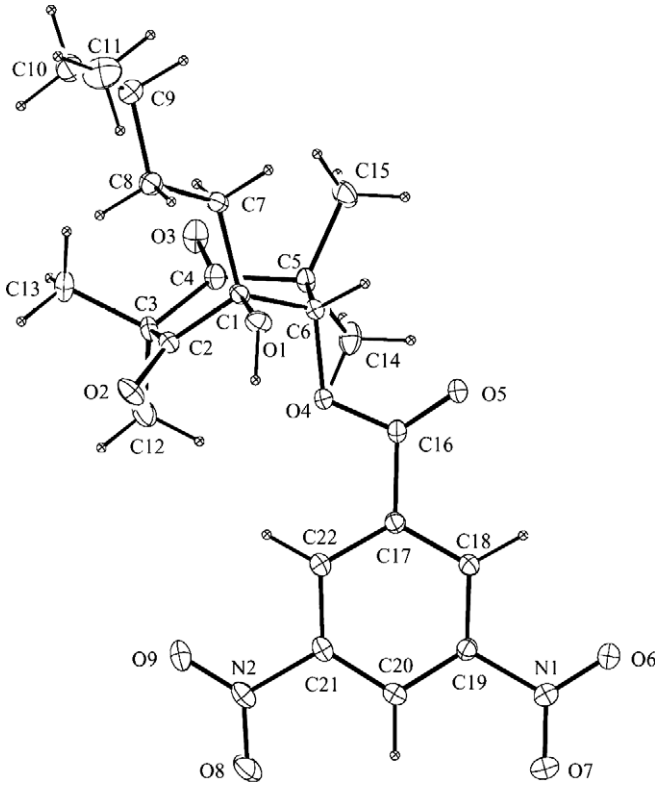


Fig. 1. X-ray image of 6-(3,5-dinitrobenzoyl)-triumphalone.

major component (>90% by NMR). After subsequent isolation on reverse phase HPLC using acetonitrile and water, both containing trifluroacetic acid (0.05%), and NMR analysis in $CDCl_3$, some 24 months after the initial work, triumphalone was the minor component (~30% by NMR) and another closely related compound had formed. While GCMS on a 5% phenyl column resolved these two components a Carbowax capillary column did not. The respective LRI data for the two compounds on a DB-5 column are 1810 for compound **1** and 1744 for compound **2**.

The additional compound exhibited the same molecular weight and empirical formula as **1**. The mass spectrum of (**2**) showed 270 (M^+, 1%), 252 (0.5), 242 (5), 246 (2), 197 (3), 193 (92), 171 (37), 155 (5), 152 (3), 137 (910), 113 (27), 99 (15), 101 (55), 83 (26), 71 (45), 55 (928), 43 (100).

The results of an NMR study of a mixture of **1** and the new compound in a 2:3 ratio allowed all proton and carbon resonances to be identified (Table 1) and clearly showed that it was structurally closely allied to **1**. From the HMBC spectrum a notable distinction was that both pairs of geminal dimethyls exhibited ^3J couplings to one carbonyl (δc 221.7) but neither had any interaction with the second carbonyl (δc 213.2). The oxymethine proton (δ 4.78) had to be placed adjacent to one gem-dimethyl because of ^3J couplings to both methyl carbons (δc 20.1, 26.9). On this basis the structure of this compound could be assigned as 1-pentyl-1,3-dihydroxy-4,4,6,6-tetramethylcyclohexa-2,5-dione (**2**), to which we have assigned the trivial name isotriumphalone.

A series of 1D nOe experiments established relative stereochemistry to be comparable to that of **1**, with the two hydroxyl groups *cis*. While no direct evidence is available it seems very likely that **2** will have the same absolute stereochemistry as **1**.

Isotriumphalone (**2**) would appear to be the product of a 1,2-shift of the pentyl group to the adjacent (protonated) carbon of triumphalone. Assuming the diol has the same boat-like conformation as this derivative, the X-ray showed that triumphalone is perfectly set up for the 1,2 shift of the pentyl group to the adjacent (protonated) carbonyl. Whilst 1,2-alky shift are well documented in terpene chemistry they have not been observed in published literature on β-triketones because it is the unique α-ketol structure of triumphalone with the appropriate stereochemistry promotes the facile pentyl migration.

2.1. Chemotaxonomy

β-Triketones have been detected in the essential oils of a number of endemic Australian Myrtaceae of the genera *Backhousia* (Brophy et al., 1995), *Eucalyptus* (Hellyer, 1968; Brophy and Boland, 1990; Brophy et al., 1991; Bignell et al., 1997; Van Klink et al., 1999; Brophy and Southwell, 2002), *Corymbia* (Van Klink et al., 1999), *Leptospermum* (Brophy et al., 2000) and *Xanthostemon* (Brophy et al., in press). In the genus *Melaleuca* (approx. 270 species, over 210 examined) they have previously only been found in *M. cajuputi* ssp. *platyphylla* (Brophy and Doran, 1996) and *M. nanophylla* (Brophy, 1999). Triumphalone is a unique variation on the β-triketone structure dominated by the 1,3- and 1,3,5-oxygenation patterns.

3. Experimental

3.1. General

IR spectra were obtained neat on a Perkin–Elmer 1600 FTIR spectrometer. NMR spectra were recorded in $CDCl_3$ on Bruker DMX-500 and DPX-400 spectrometers. High resolution mass spectra were recorded under FAB conditions on a Kratos Concept ISQ mass spectrometer.

3.2. Plant material

The plant material analyzed was collected at Canberra from greenhouse-grown plants derived from seed collected by I. Cowie from the Victoria River Gorge and is vouchered by the collections *Cowie & Mangion* 7321, 7325, 7327, specimens of which are deposited in the herbaria CANB and DNA.

3.3. Extraction and identification of compounds

The leaf oils were isolated by hydrodistillation with cohobation for 8 h as previously outlined in Brophy et al. (1991).

3.4. Identification of components

Analytical gas chromatography (GC) was carried out on a Shimadzu GC17 gas chromatograph. A WCOT DB-Wax column [60 m × 0.5 mm, film thickness 1 mm] was used, programmed from 50°C to 225 °C at 3 °C/min with helium at 3.5 mL/min as carrier gas. GC integrations were performed on a SMAD electronic integrator without the use of correction factors. GC/MS was performed on both a VG Quattro mass spectrometer operating at 70 eV ionization energy (the column used was DB-Wax [60 m × 0.32 mm, film thickness 0.25 mm] programmed from 35 °C to 220 °C at 3 °C/min, with helium at 35 cm/s as carrier gas) and a Shimadzu QP5000 instrument equipped with a DB-5 column [30 m × 0.25 mm, film thickness 0.25 mm]. The latter column was programmed from 35°C to 250 °C at 5 °C/min, helium carrier gas flow rate was 30 cm/s. Compounds were identified by their identical GC retention times to known compounds and by comparison of their mass spectra with either known compounds or published spectra (Joulain and König, 1998; Adams, 2001; Swigar and Silverstein, 1981; Stenhagen et al., 1974; Heller and Milne, 1978, 1980, 1983).

3.5. Isolation of triumphalone (*1*)

Triumphalone was isolated, in approximately a 10:1 ratio with isotriumphalone by chromatography of the

crude steam distillate on silica gel. Elution with pentane removed the hydrocarbons, while increasing amounts of CH_2Cl_2 in pentane gave the oxygenated sesquiterpenes. Triumphalone was eluted with 30–80% CH_2Cl_2 in pentane.

Triumphalone was also isolated by semi-preparative HPLC on an Agilent 1100 HPLC system equipped with a quaternary pump, column oven (40 °C) and a diode array detector (collecting spectra in the range 190–900 nm). Separation was achieved on a Zorbax (USA) SB-C18 column (9.4 mm × 250 mm, 5 μm) using an acetonitrile (0.05% TFA) and water (0.05% TFA) gradient, 50–95% acetonitrile (TFA) over 15 min, flow rate 2.5 ml/min. Fractions were collected manually and identity confirmed by GC–MS. Under these conditions triumphalone eluted at 11.2 min.

3.6. Crystallography[1]

3.6.1. Crystal data

$C_{22}H_{28}N_2O_9$, M 464.5, m.p. 109–110 °C, orthorhombic, space group $P2_12_12_1$, $a = 8.434(2)$, $b = 14.537(2)$, $c = 19.338(4)$ Å, $V = 2370.9(8)$ Å^3, $D_c = 1.30$ g cm^{-3}, $Z = 4$, $\mu_{Mo} = 0.098$ mm^{-1}. Crystal size 0.30 by 0.15 by 0.12 mm, $2\theta_{max} = 50°$. The number of reflections was 1615 considered observed out of 2373 unique data. Final residuals R, R_w were 0.038, 0.043.

3.6.2. Structure determination

Reflection data were measured with an Enraf-Nonius CAD-4 diffractometer in $\theta/2\theta$ scan mode using graphite monochromated molybdenum radiation ($\lambda = 0.71073$ Å). Reflections with $I > 2\sigma(I)$ were considered observed. The structure was determined by direct phasing and Fourier methods. Hydrogen atoms were included in calculated positions and were assigned thermal parameters equal to those of the atom to which they were bonded. Positional and anisotropic thermal parameters for the non-hydrogen atoms were refined using full matrix least squares. Reflection weights used were $1/\sigma^2(F_o)$, with $\sigma(F_o)$ being derived from $\sigma(I_o) = [\sigma^2(I_o) + (0.04I_o)^2]^{1/2}$. The weighted residual is defined as $R_w = (\sum w\Delta^2/\sum wF_o^2)^{1/2}$. Atomic scattering factors and anomalous dispersion parameters were from International Tables for X-ray Crystallography (Ibers and Hamilton, 1974). Structure solution was by SIR92 (Altomare et al., 1994) and refinement used RAELS (Rae, 1996). ORTEP-II (Johnson, 1976) running on an eMAC was used for the structural diagram, and an eMAC computer was used for calculations.

[1] CCDC 299088 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336 033, deposit@ccdc.cam.ac.uk).

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