A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers

DOAJ Seal

Arkivoc **2022**, part iv, 0-0
to be inserted by editorial office

Phytochemical composition of *Denhamia obscura* (A. Rich.) Meisn. Ex Walp. root bark, seeds and leaves

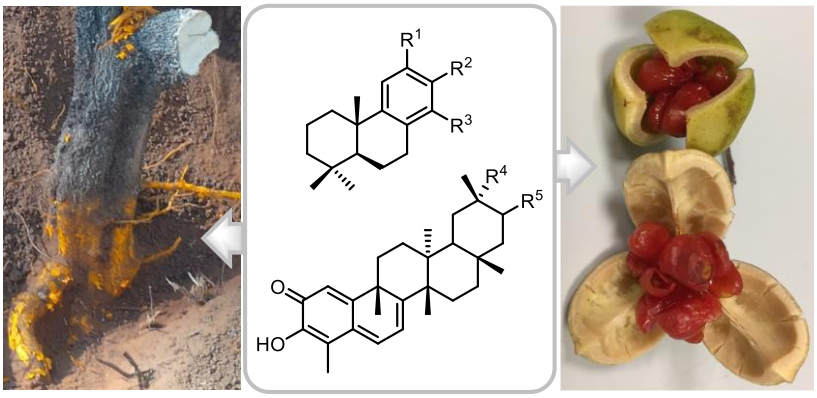
Matheus Carpinelli De Jesus,[a] Johanna Wapling,[b] Eunice Kiem,[a] Tegan Stockdale,[a] Jacob J. Whittaker,[a] Jack K. Clegg,[a] David Leach,[c] Taylah Church,[b] Raelene Collins,[b] Greg Leach,[b] David Hewitt,[d] James J. De Voss,*[a] and Joanne T. Blanchfield*[a]

[a] *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, 4072, QLD, Australia*
[b] *Menzies School of Health Research, Charles Darwin University, Darwin, NT, Australia*
[c] *Integria Healthcare (Australia), Brisbane, QLD, Australia*
[d] *Wild Orchard Kakadu Plum, Deleye Outstation, Nemarluk, NT, Australia*
Email: m.carpinellidejesus@uq.edu.au

Received mm-dd-yyyy **Accepted Manuscript** mm-dd-yyyy **Published on line** mm-dd-yyyy
Dates to be inserted by editorial office

Abstract

An investigation of the chemical composition of the root bark, seeds and leaves from the Australian plant, *Denhamia obscura* was carried out. Despite the traditional medicinal use of this plant by Indigenous communities in Australia and the comprehensive studies of other *Denhamia* species, the phytochemical profile of this plant has not been reported previously. Twelve known pentacyclic triterpenes, as well as seven abietane compounds were characterised. Two of the abietane compounds are new natural products, previously reported as synthetic compounds, obscurol and 13-methoxy-sempervir-6-ene. Friedelin was isolated from the root bark and leaves, with X-ray crystallography utilised to support its absolute configuration. The chemical compounds identified in this profile are consistent with other species now phylogenetically associated with the *Denhamia* genus.



Keywords: Natural Products, *Denhamia obscura*, Traditional medicine, abietane, triterpene, X-ray crystallography

Introduction

The plant genus *Denhamia* (family Celastraceae) is endemic to Australia and the Pacific Islands region.[1] The genus contains 15 recognised species (to date) including eight species previously included in the genus *Maytenus*.[1,2] Only three studies have endeavoured to characterise the composition of two plant species from this genus, *Denhamia celastroides* and *Denhamia pittosporoides.* These studies focused on identifying the compounds found in the leaves of these plants.[3-5] The first study into the composition of *D. celastroides* leaves was performed in 2015 by Levrier and Davis et al. who identified eight compounds named denhaminols A–H.[4] These compounds are esters of polyhydroxy sesquiterpenes, all belonging to the dihydro-β-agarofuran compound family (Figure 1). While the compounds all shared a similar core structure, they were functionalised with acetate, benzoate, tigliate and/or cinnamate esters on various hydroxylated positions. These compounds inhibit growth of the human prostate carcinoma cell line LNCaP with denhaminol A and G the most effective.

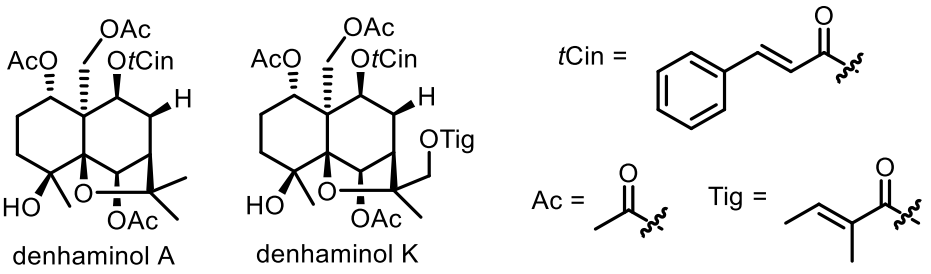


Figure 1. Structure of two dihydro-β-agarofurans isolated from *D. celastroides*.[3]

In 2018, Davis' group published a second paper[3] on the composition of *D. celastroides*. This study reported the isolation of new denhaminols K–N in addition to those identified in the initial work. These were minor compounds that evaded detection during the previous studies. In this study, Davis et al. also saponified a portion of the denhaminol A extracted to generate the core sesquiterpene lacking ester moieties, to assess the effect of the esters on uptake and cell growth inhibitory activity. Leucine transport in the LNCaP cells was inhibited by all denhaminols A–N, with denhaminol K being the most effective while the sesquiterpene aglycone exhibited no biological effect.

The third study investigated *D. pittosporoides.*[5] Following the same experimental protocol[4] denhaminol I and J were isolated alongside four other structurally related compounds corresponding to a total of 0.3% of dry weight.

A distinctive characteristic of *Denhamia obscura*, investigated here, is the presence of a bright yellow layer in the root bark which is highly flammable and changes to a yellow-orange color after exposure to air. The function of such a pigmented layer in an underground organ raises some ecological questions. For example, does the layer provide some protection of the root from herbivores or microbial pathogens? An understanding of the chemistry of this layer will assist in understanding its function. The species also has a bright red fleshy aril surrounding the seed. Fleshy arils normally function as an attractant to seed dispersers by providing a food reward. The presentation of the seed in the open fruit capsule while still hanging on the tree suggests that birds are the targeted dispersal agent for the seeds. It is of interest to know if the aril pigmentation is based on the same chemistry as the root bark pigmentation given the very different functions proposed for the two materials, *i.e.*, herbivore/pathogen deterrent and seed disperser attractant.

Denhamia obscura is a species endemic to Australia, which has been used by Aboriginal and Torres Strait Islander communities for centuries as a traditional medicine. Pharmacopoeias report that chewing the bright yellow-orange root bark alleviates toothaches,[6] and the aqueous extract from boiled leaves is used in the treatment of respiratory ailments.[7] Hyland and colleagues report aphrodisiac effects resulted from the consumption of the root bark with limited details provided.[8] The Tiwi people of the Northern Territory also attribute the yellow coloration as a "good luck" charm in various endeavours such as travel or significant life events.[7] The characterisation of the natural products present in the root bark and leaves of *D. obscura* is the focus of this study, since these are parts of the plant used in traditional medicine. Alongside the phytochemical characterisation of the root bark and leaves, assays were conducted on the crude extracts to evaluate the antimicrobial potential of the root bark extracts. On the other hand, compounds isolated from the leaves shed light on the biosynthetic pathway by which this plant produces triterpene natural products.

Results and Discussion

Root bark

Denhamia obscura was collected from three locations in the Top End of the Northern Territory, Australia. Samples were dried, milled and transported to the University of Queensland, where they were suspended in methanol and sonicated to increase the efficiency of the extraction process. The methanolic extract was then partitioned with hexane. Water was added to the methanol partition, which was further partitioned with chloroform. These partitioning steps yielded three extracts for analysis: hexane; chloroform; and aqueous methanol. The hexane and chloroform extracts were subjected to normal phase high performance liquid chromatography (NP HPLC) using a mixture of hexane and ethyl acetate as eluting solvents. The aqueous methanol partition was fractionated using reverse phase (RP) HPLC using acetonitrile/water mixtures as the eluting solvent. Fractionation by NP HPLC of the chloroform partition yielded abietanes **1-7** and triterpenes **8**-**14** (Figure 2), with known natural products characterised as ferruginol (**1**), maytenoquinone (**2**), dispermol (**4**), 12-hydroxytotarol (**5**), sugiol (**6**), pristimerin (**8**), celastrol (**9**), tingenone (**10**), iguesterin (**11**), friedelin (**12**), multifluorenol (**13**), glutinol (**14**). Abietanes **3** and **7** were identified by a combination of NMR spectroscopy and mass spectrometry from simple mixtures. Fractionation of the methanolic partition only yielded compounds **2**, **3**, **8**, **12** and **14** already identified in the other extracts.

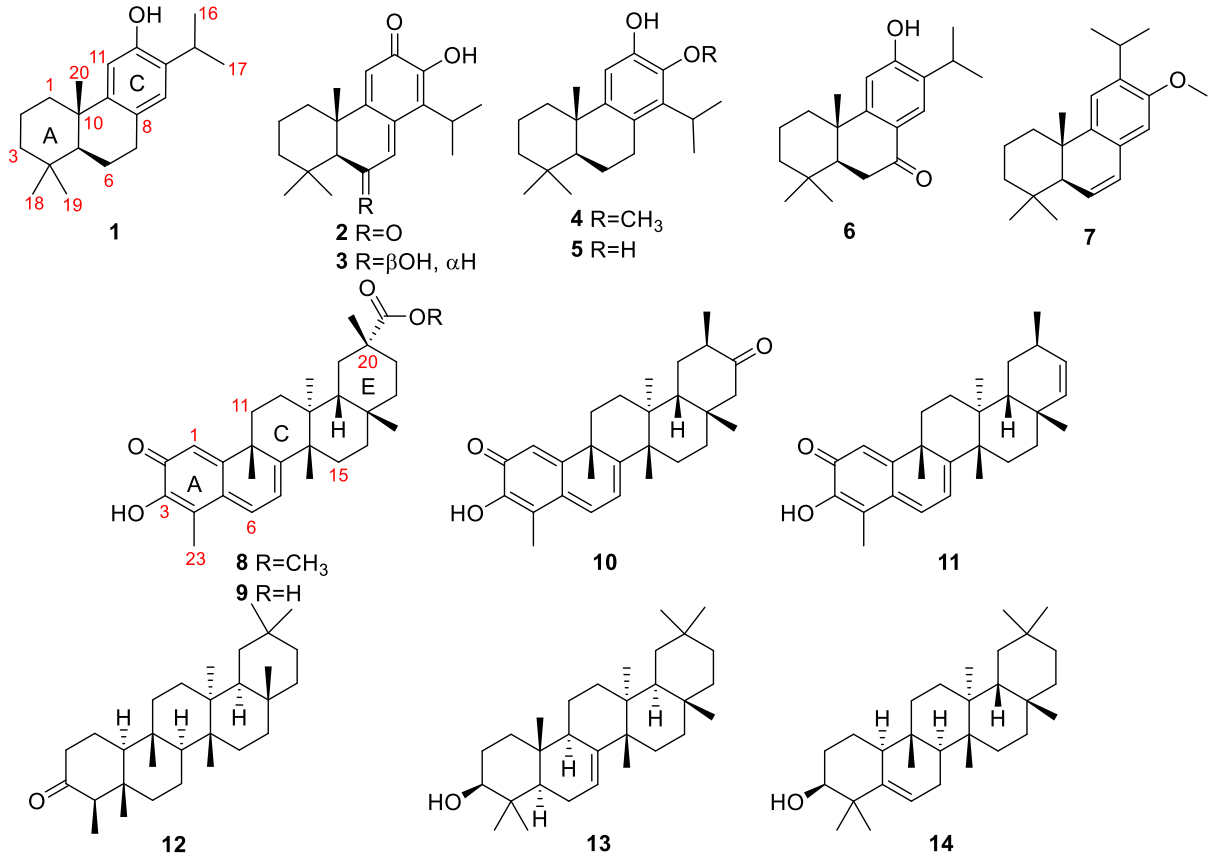


Figure 2. Compounds isolated from *D. obscura* root bark.

Recent phylogenetic research has reclassified *Maytenus* species, common in Australia and Southern Pacific Islands, as belonging to the *Denhamia* genus (Figure 3).[2] The presence of abietane-like compounds and triterpenes in this *D. obscura* extract is consistent with these natural products (compounds **2**, **6**, **9-12**) having previously been isolated from plants formerly identified as *Maytenus* but now reclassified as belonging in *Denhamia*.[9-11] The chemistry therefore appears to support this taxonomic realignment.

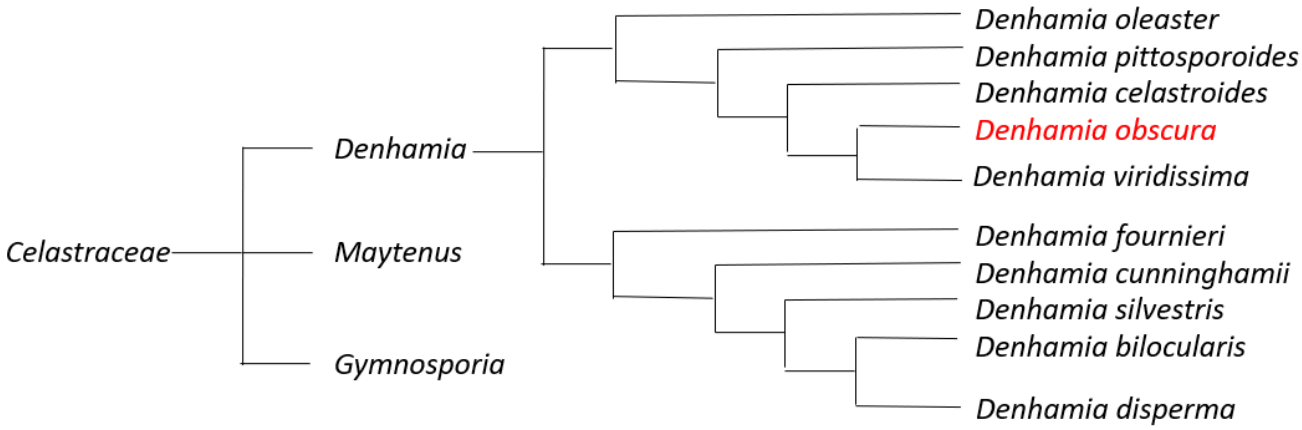


Figure 3. Phylogenetic distribution of *Denhamia* genus based on works by Mckenna[1] and Simmons.[2]

Compounds **1**, **2**, **5**, **6**, **8-14** have been previously isolated as natural products with some also being the focus of synthetic studies,[12-15] providing sufficient characterisation data for comparison. The synthesis of **3** has been reported[10] but there has been no previous reports of its occurrence as a natural product.

The abietane skeleton gives rise to characteristic ^{1}H NMR signals including five methyl signals (δ_C 21.0–33.0): three singlets corresponding to the methyl groups on ring A at C18, C19 and C20; and two doublets corresponding to the methyl groups of the isopropyl group attached to the aromatic C ring, positions 16 and 17. Both of these signals in the COSY spectrum exhibit correlation to the septet signal at approximately 3.00 ppm (δ_H 3.11 for ferruginol **1**).

The NMR spectra of compound **3** exhibited peaks analogous to the characteristic ones described above: three methyl singlets in the ^{1}H NMR at 1.13, 1.18 and 1.21 ppm corresponding to H-18, 19 and 20, respectively; and two methyl doublets for H-16 and 17 at 1.36, 1.33 ppm with a vicinal coupling constant of 7.0 Hz, assigned to the isopropyl methyl groups. The latter proton signals exhibited coupling to the septet signal at 3.11 ppm (H-15), further supporting the presence of an isopropyl group. The methyl signals at 1.36 and 1.33 ppm show three bond correlation to a signal at 161.6 ppm (C-14), and four bond correlations to a signal at 135.8 ppm (C-8) and 126.2 ppm (C-13) in the HMBC spectrum. Another characteristic feature of unsaturated abietanes is the appearance of low field proton signals at 6.88 and 6.27 ppm corresponding to H-7 and 11 of the extended conjugated quinone methide system present in **3**. The correlation between H-11 and C-7 that was observed in the HMBC supported the presence of this moiety. The hydroxyl moiety attached to C-13 was assigned to a singlet at 6.95 ppm. An interesting feature of compound **3** was the appearance of a broad doublet at 4.69 ppm, which exhibited a 10 Hz coupling to the signal at 1.89 ppm, assigned to the bridgehead H-5. These data suggested that in **3**, C6 (δ_C 69.9) is an sp^3 hybridised carbon bearing an hydroxyl group, H-6 appears at 4.69 ppm and H-5 and H-6 are *trans*-diaxial. This is consistent with the *trans* A,B-ring junction observed in the other abietanes isolated and with the hydroxyl group at C-6 ring equatorial. Thus, compound **3** was characterised as 6,13-dihydroxytotar-6,8(11),13-triene. This compound has not previously been isolated as a natural product, but has been reported synthetically;[12] the NMR data observed here matched that reported, and compound **3** is hereafter referred to as obscurol.

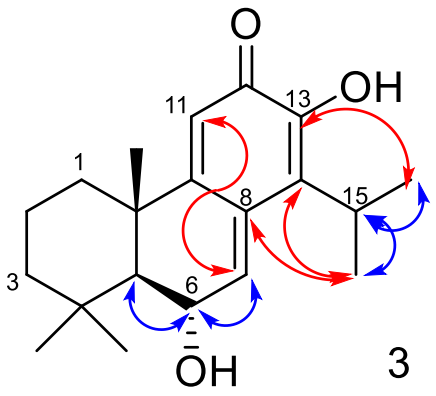


Figure 4. COSY (blue) and HMBC (red) correlations discussed in the assignment of obscurol (**3**).

Compound **7** was identified as a minor (~11%) component in a mixture with maytenoquinone (**2**). The compound was characterised initially through its diagnostic signals and then structural elucidation was completed using 2D NMR techniques to arrive at a proposed structure. The overall distribution of signals in the ^{1}H spectra suggested the abietane-like nature of the core, with three singlets at 1.01, 1.04, 0.94 ppm

corresponding to H-18, 19 and 20, respectively, and two downfield singlets at 6.71 and 6.61 ppm assigned to H-11 and H-14. These assignments were supported by HSQC correlation of these signals to carbons with methyl (δ_C 20.8, 21.6, 33.1) and aromatic character (δ_C 114.0, 131.0), respectively. The COSY spectrum provided evidence for the isopropyl moiety due to the correlations between the doublets at 1.34, 1.32 ppm (H-16, H-17) and the septet at 3.06 ppm (H-15). These proton signals were then assigned to their respective carbons via HSQC (27.2, 21.3, 21.4 ppm, C-15, 16, 17, respectively). HMBC correlation between the methoxy ^{1}H NMR signal (3.75 ppm) and an aromatic carbon signal tentatively assigned as C-13 (147.3 ppm) supported the presence of a methyl ether in ring C. Other signals assigned based on the established abietane core were the signals associated with ring A (C-1-3). These assignments were possible by combining the HSQC data, which associated the methylene signals with carbon signals, and HMBC correlations between methyl signals for C-18–20 and these sp^3 hybridised carbons. Finally, the signals at 5.88 and 6.84 ppm each observed as a doublet of doublets were assigned to H-6 and H-7 of ring B. Each showed a vicinal coupling of 10 Hz, as expected for a *cis* coupling. COSY correlations suggested that both H-6 and H-7 coupled to H-5. The magnitude of the coupling constant (2.9 Hz) between H-5 and H6 is consistent with a dihedral angle of approximately 92° (Chem 3D Ultra 20.0.0.41, PerkinElmer, MM2 minimised energy), congruent with H-5 occupying the axial position which is the stereochemistry observed in other abietane compounds isolated from this plant. The smaller coupling (^4J = 3 Hz) exhibited by H-7 is likely a 'w'-coupling to H-5 due to the allylic nature of this 'H-C-C-C-H' segment of the structure. Since very few HMBC correlations were observed, with none connecting rings B and C, the structure could have been 6,7-dehydroferruginol methyl ether, reported by Cordova-Guerrero and colleagues,[16] or structure **7** in Figure 5. The NMR data from the previously reported natural product did not align with the compound indicated here.The structure of **7** was published as part of a synthetic protocol,[17] but no NMR or other characterisation data was given. Therefore, this is the first publication of the NMR data as well as the first report of it as a natural product, and it is named 13-methoxy-sempervir-6-ene (**7**), based on the similarity of the structures to sempervirol, the target natural product in the synthetic paper which reported these compounds.

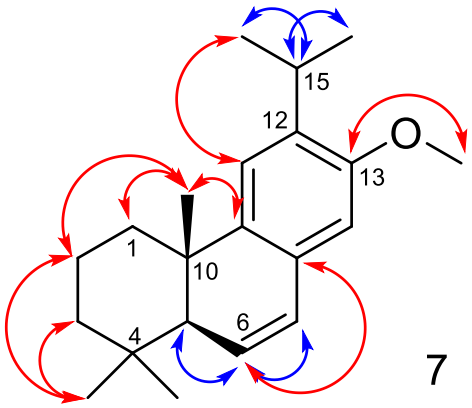


Figure 5. Struture for 13-methoxy-sempervir-6-ene (**7**) with COSY (blue) and HMBC (red) correlations.

The phytochemical characterization discussed above was conducted on a sample collected from Thorak Road (see experimental for coordinates). Root bark samples were also collected from Buffalo Creek and Deleye Outstation (see experimental for coordinates), during different seasons. For comparison, a methanolic extract (21.1 mg) of samples from each collection site, was suspended in aqueous methanol (50%) and screened with the same method and conditions on LC-PDA-MS (10 μL), GCMS (2 μL) and ^{1}H NMR (CD_3OD, 32

scans). Compounds were identified by matching retention time, UV-vis and ESIMS data. Once those compounds were identified, it was possible to compare the relative abundance of compounds qualitatively, based on UV-detected chromatogram intensities and total ion count. Compounds **4**, **5** and **7** were observed inconsistently: **7** was not observed in the Deleye outstation sample, **4** and **5** were not observed in the Buffalo creek sample, while **1**-**3**, **6**, **8**-**11** were observed across all three samples. These samples were collected during different seasons, which may impact the phytochemical composition. Alternatively, there may be some geographical variation in the phytochemical composition of the bark.

With the phytochemical characterisation complete, it was possible to determine which compound contributed to the color of the bark. Maytenoquinone (**2**), λ_{max} 345 nm, the most abundant abietane and found in all samples provides the yellow tones of the root bark. Pristimerin (**8**) (λ_{max} 438 nm), celastrol (**9**) (λ_{max} 438 nm) and other triterpenes provided a deep red hue, which combine to enrich the vivid yellow coloration of the root bark.

Members of the abietane family of compounds, *e.g*., **1**-**7**, and pentacyclic triterpenes with extensive conjugation, *e.g*., **8-16**, have been identified in various studies as exhibiting anti-inflammatory, anti-cancer, and some cytotoxic activity.[18] The root bark crude methanolic extract from the Thorak Road site was screened for antimicrobial activity against a panel of microbes including Gram negative bacteria [*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25668)], gram positive bacteria [*Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615)] and the yeast *Candida albicans* (ATCC 90029). Antimicrobial activity was examined at a single concentration (20 mg/mL) in a broth microtiter method based on Clinical Laboratory Standards Institute (CLSI) standards for Antimicrobial Susceptibility testing.[19,20] Antimicrobial activity was defined as a reduction of 80% or greater in growth as compared to the control. The crude extract derived from the Thorak Road sample demonstrated antimicrobial activity against *S. aureus*. This data is in agreement with the previously reported effects of abietane and triterpenes against *S. aureus* and *Propionibacterium acnes*.[21] The demonstration of the antimicrobial activity of the root bark compounds supports the hypothesis that these compounds may provide some protection against microbial pathogens in the soil.

Seeds

An analogous process of extraction and fractionation was carried out on *D. obscura* whole seeds, which contained compounds **1-4**, **6**, **8-10**, **13** and **14**, some of which are responsible for the red coloration of the fleshy aril covering the seeds. The difference in coloration between the root bark and the aril material stems from the variation in concentration of compounds, with a higher abundance of compounds **8** and **9** compared to compound **2** in the aril material. The identification of these compounds was carried out via LC-PDA-MS by comparison of retention time, UV-vis absorbance and ESIMS data to authentic compounds isolated from the root bark.

Characterization of the remaining components in the seed extract was achieved by GCMS. This led to the tentative characterization of eleven compounds by comparison to mass spectrometric libraries. Tentative identification was assigned to compounds presenting a mass spectrum similarity over 93% in at least two of three libraries (NIST17, FFNSC and WIST29). These compounds were; heptanal, *E*-2-decenal, 2,4-decadienal (*E,E*) and (*E,Z*), methyl of eicosanoate, palmitic acid, methyl 9,12-octadecadienoate, methyl 9-octadecanoate, methyl stearate, linoelaidic acid and stearic acid.

Leaves

The preliminary GCMS screening of an *n*-hexane extract of *D. obscura* leaves did not indicate the presence of denhaminols but four pentacyclic triterpenes were identified as significant components of the extract: lupen-3-one (**15**); germanicone (**16**); β-amyrone (**17**); and friedelin (friedelan-3-one) (**12**) (Figure 2). Extraction of the leaves with more polar dichloromethane resulted in the isolation of **12**, **15**-**17** as well as glutinol (**14**) and lupeol (**18**), while extraction with methanol produced an extract rich in α-glucose, β-glucose and β-xylose.

Friedelin (**12**) was easily isolated due to its abundance and proclivity to crystallise. This compound was the most prevalent in the extract of *D. obscura* leaves. In 1955, the absolute configuration of friedelin was determined by Corey and Ursprung through a lengthy series of degradation studies.[22] To date, all friedelin isolated from natural sources has exhibited the same negative optical rotation, with slight fluctuations in the reported values due to differences in concentration and temperature. The crystallised sample of friedelin in this work exhibited an [a] = -12° (c = 0.4 in chloroform, at 24 °C and 589 nm). All naturally occurring friedelin samples have therefore always been assigned the absolute configuration consistent with the original 1955 determination. The low temperature crystal structure of **12** obtained here (Figure 6) established the absolute configuration, which was consistent with the 1955 assignment as 4*R*, 4a*S*, 6a*S*, 6b*R*, 8a*R*, 12a*R*, 14a*S*, 14b*S*.[23-25]

1D and 2D NMR analysis of the crystallised friedelin allowed for the complete assignment of both the ^{1}H and ^{13}C NMR spectra of the compound. Complete assignment of the ^{13}C NMR spectrum of friedelin has been published,[26] but no such data has been published for the ^{1}H NMR spectrum (Table 1).

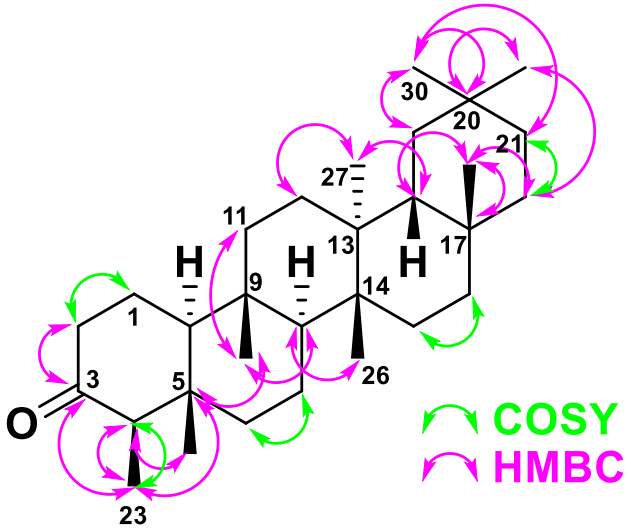


Table 1. Assignment of ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of friedelin (**12**) in chloroform-*d* and literature ^{13}C NMR (100 MHz) data.

		^{1}H NMR (*J* = Hz)	^{13}C NMR	^{13}C NMR Lit.[26]
1a	CH_2	1.97 (ddt, 13.0, 7.1, 2.0)	22.3	22.3
1b		1.68 (dd, 13.0, 5.1)		
2a	CH_2	2.39 (ddd, 14.0, 5.1, 2.0)	41.5	41.5
2b		2.30 (ddd, 14.0, 7.2, 1.2)		
3	q		213.2	213.2
4	CH	2.25 (q, 7.1)	58.2	58.2
5	q		42.1	42.2
6a	CH_2	1.75 (dt, 14.0, 2.9)	41.3	41.3
6b		1.29-1.26 (m)		

7a	CH_2	1.51-1.49 (m)	18.2	18.3
7b		1.37 (q, 2.7)		
8	CH	1.39 (s)	53.1	53.1
9	q		37.4	37.5
10	CH	1.54 (brd s)	59.5	59.5
11a	CH_2	1.46 (dd, 8.4, 4.4)	35.6	35.6
11b		1.28-1.25 (m)		
12	CH_2	1.34 (s)	30.5	30.5
13	q		38.3	38.3
14	q		39.7	39.7
15	CH_2	1.48* (br s)	32.4	32.4
16a	CH_2	1.62-1.58 (m)	36.0	36.0
16b		1.53 (s)		
17	q		29.9	30.0
18	CH	1.57-1.55 (m)	42.8	42.8
19a	CH_2	1.38-1.34 (m)	35.3	35.4
19b		1.21 (dd, 14.0, 5.9)		
20	q		28.2	28.2
21	CH_2	0.94 (s)	32.7	32.8
22	CH_2	1.48* (brd s)	39.2	39.2
23	CH_3	0.88 (d, 6.8)	6.8	6.8
24	CH_3	0.72 (s)	14.6	14.7
25	CH_3	0.87 (s)	17.9	18.0
26	CH_3	1.01 (s)	20.2	20.3
27	CH_3	1.05 (s)	18.6	18.7
28	CH_3	1.18 (s)	32.1	32.1
29	CH_3	1.00 (s)	35.0	35.0
30	CH_3	0.95 (s)	31.8	31.8

*Overlapping signals in the ^{1}H NMR

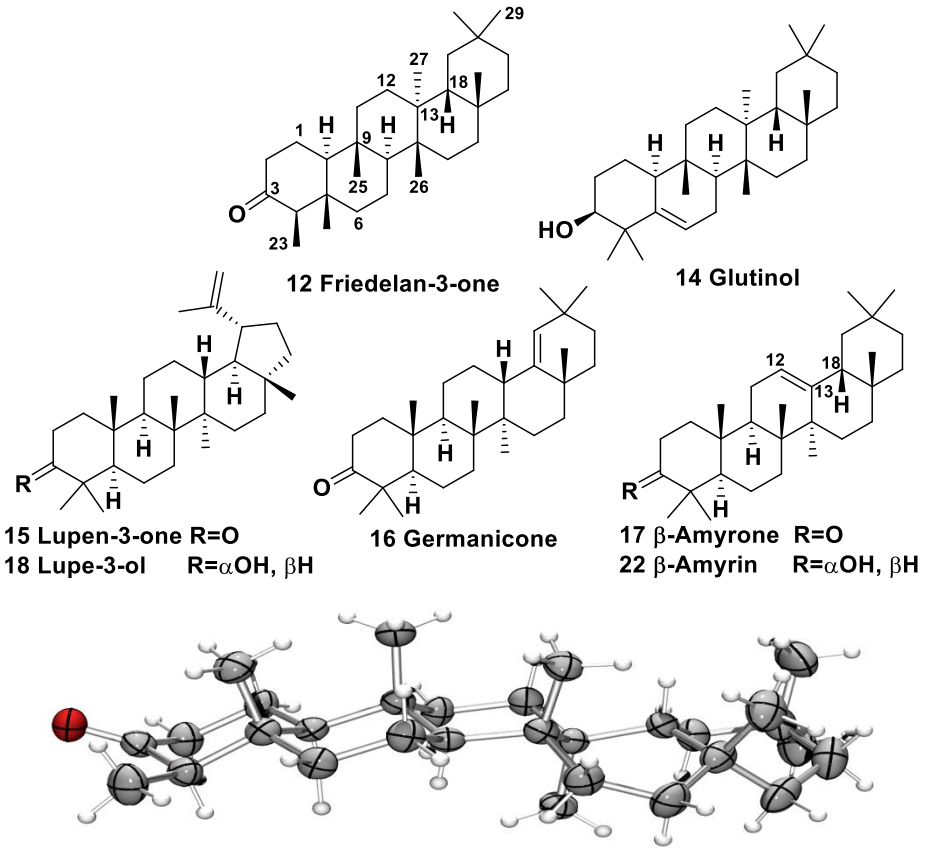


Figure 6. Pentacyclic triterpenes **12**, **14-18** isolated from *D. obscura* leaves, with ORTEP of the crystal structure obtained for **12** (CCDC number: 2150530) and structure for related compound **22**.

Other triterpenes were obtained as mixtures but were still able to be confidently identified by high field NMR and GCMS analysis. Lupenone (**15**) was obtained either as a mixture with friedelin (**12**) or with germanicone (**16**) and β-amyrone (**17**), while lupeol (**18**) was only isolated as a mixture with glutinol (**14**). The characterisation of these compounds was possible due to key resonances associated with the olefinic and methyl group. The olefinic CH_2 group characteristic of lupenone (**15**) and lupeol (**18**) is observed as signals at 4.63 (br s, H-29b) and 4.50 (br s, H-29a) ppm in the ^{1}H spectrum of **15** and at 4.67 and 4.57 ppm in the case of **18**. Compounds **15** and **18** were identified in different fractions, characterised by their distinct ^{1}H NMR chemical shifts and molecular weight as determined by GCMS. Germanicone (**16**) and β-amyrone (**17**) have endocyclic olefins at C-18 and C-12, respectively. The olefinic protons of these trisubstituted double bonds are observed characteristically as broad singlets at 4.88 (H-19) ppm for **16** and at 5.20 (br s, H-12) ppm for **17**. Since the GCMS (EI) fragmentation patterns of these compounds and their diagnostic ^{1}H NMR resonances were consistent with literature data, the mixtures were then analysed by HSQC allowing assignment of methyl and olefin carbon shifts that were also in agreement with literature values.[27-30]

A mixture of lupenone (**15**) and germanicone (**16**), at a 1:1.5 ratio respectively, had a measured specific optical rotation in chloroform [a] +38 (c = 0.2 at 24 °C and 589 nm). The comparable literature values (chloroform, at 24 °C and 589 nm) are [a] +37 for **16**[31] and [a] +64 for **15**.[32] The fact that the sign of the specific rotation for this mixture is consistent with these literature values suggests that the configuration of both **1** and **2** is that previously reported in the literature as 3a*R* and 4a*R*, respectively.

Compounds **12** and **15**-**17** have not previously been reported from the same plant but have been postulated to belong to the same biosynthetic pathway. The previously postulated biosynthetic pathway by

Thimmappa and colleagues[33] is shown (Figure 7) and supported here by isolation of **12** and **15**-**17** from the same plant species and indeed the same plant part.

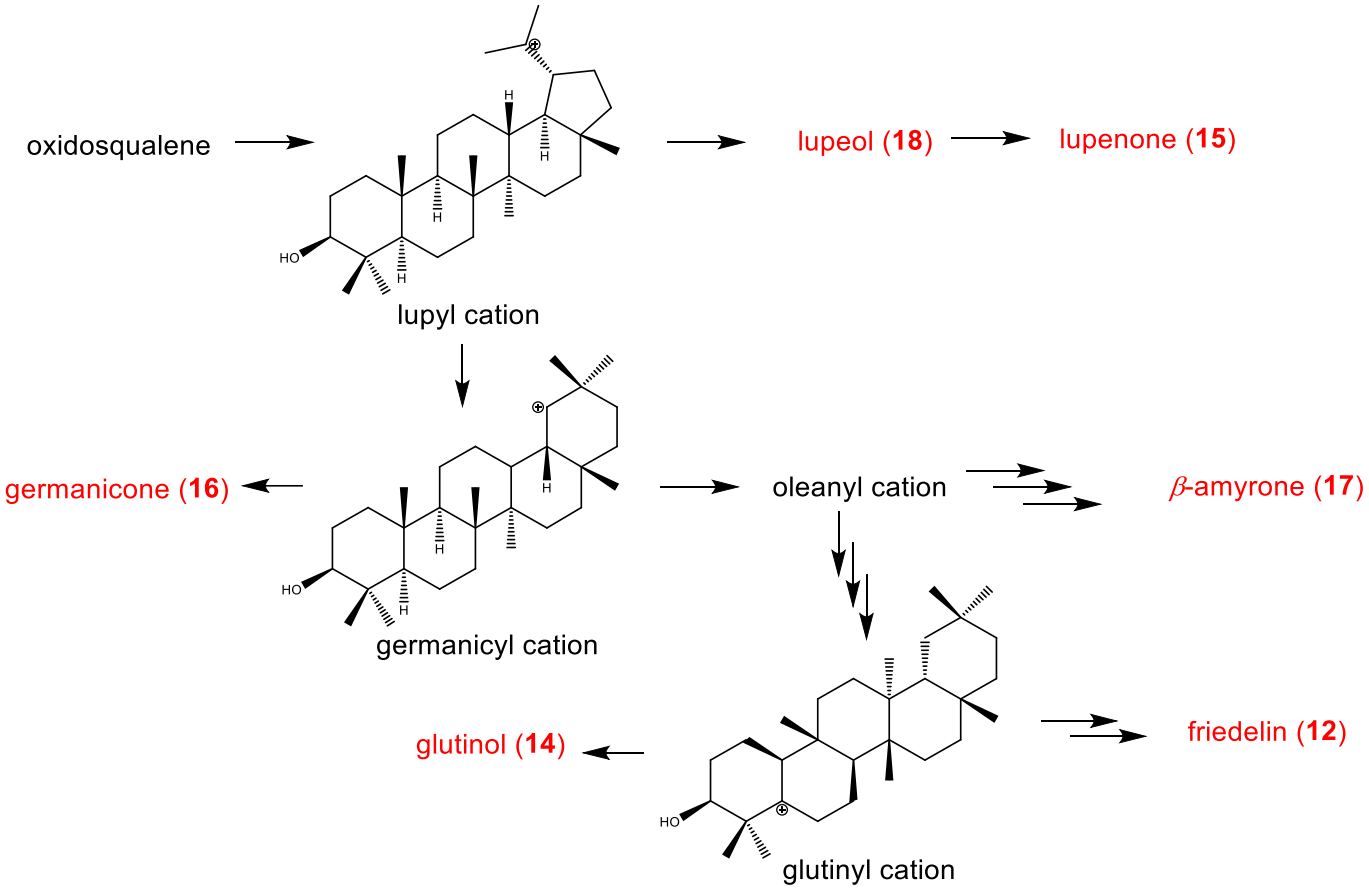


Figure 7. Biosynthetic pathway as proposed by Thimmappa et al.[33] with isolated compounds identified here shown in red.

Silva's investigation into *Maytenus gonoclada* identified lupeol (**18**), β-amyrin (**22**) and friedelin (**12**) within this Brazilian relative of *D. obscura*, with no report of the presence of germanicone (**16**).[34] A biochemical study into *Maytenus ilicifolia*,[35] characterised an oxidosqualene cyclase, dubbed friedelin synthase which produced **12** only; a mutant of this enzyme yielded β-amyrin and friedelin (**12**). Since germanicone (**16**) and β-amyrin (**22**) differ only in the position of their double bond and friedelin **12** and glutinol **14** differ in the position of a methyl group and an unsaturation we postulate that all of these compounds may either arise from a non-specific triterpene cyclase or possibly different but related synthase enzymes.

Conclusions

Abietanes **1-7** and pentacyclic triterpenes **8-14** were isolated and elucidated from the methanolic extract of *D. obscura* root bark, with compounds **1-4**, **6**, **8-10**, **13**, and **14** also isolated from the extract of the seeds. Compound **3** was characterised and compound **7** was tentatively characterised as new natural products and named obscurol and 13-methoxy-sempervir-6-ene, respectively. With the traditional use,[36] the antimicrobial results presented here and previously studied activity of maytenoquinone (**2**), sugiol (**6**), pristimerin (**8**) and

celastrol (**9**),[11] suggest the logical progression to this research is biological testing to ascertain other medicinal uses of this plant.

Qualitative comparison of the phytochemical composition across three different collection sites, provided data on some diagnostic compounds associated with *D. obscura* and highlighted some differences. For a more conclusive evaluation of phytochemical diversity, samples would need to be collected across multiple seasons and locations for statistically significant conclusions to be drawn.

It is suggested that the bright yellow of the root bark is the result of the particular composition of compounds that provide the best defence against soil borne herbivores/pathogens. The red coloration in the aril on the other hand would seem to be positively selected for because it is a signal to seed dispersers that a food source is available. Red is typically seen as a color indicating ripeness and hence palatability of fruit or seeds.

Compounds **12** and **14**-**21** were isolated from the leaves of *D. obscura* and characterised including support of the 1955 absolute configuration assignment for friedelin (**12**) by X-ray crystallography. As the absolute configuration of all compounds isolated appear to belong to the same enantiomeric series, the suite of compounds isolated provides strong support for the previously proposed biosynthetic pathway of this suite of compounds.[33]

Experimental Section

Plant collection. Root bark specimens were collected at the following locations: Thorak Rd, Berrimah, NT (12°25’53”S, 130°57’0”E) vouchered by specimen Leach 4800; Buffalo Creek, NT (12°20’12.05”S, 130°54’5.57”E) vouchered by specimen Church 57 and Deleye Outstation, NT (13°45’21.72”S, 129°58’47.52”E,) vouchered by specimen Church 15.

Seeds were collected from Henry Wrigley Dr, Eaton, NT (12°24’01.0”S, 130°52’38.0”E) by Dr Gregory Leach and vouchered by specimen Leach 4780.

Leaves were collected at Thorak Rd, Berrimah, NT (12°25’53”S, 130°57’0”E) vouchered by specimen Leach 4800L.

All collections were authenticated by Dr Gregory Leach. All voucher specimens are deposited at the Northern Territory Herbarium in Darwin, NT.

Samples were collected under NT Parks & Wildlife permit 60819 and Northern Land Council research permit where required.

This work was conducted as part of a project reviewed and approved by Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC reference number2018-3263).

Plant extraction. Root bark shavings were oven dried (40 °C) and root bark shavings were ground (10 g), suspended in methanol (200 mL) and placed in an ultrasonic bath (30 min). The filtrate was extracted with *n*-hexane (3 × 100 mL). Water (100 mL) was added to the methanol partition remaining from the filtrate (post extraction with *n*-hexane), and the mixture was then extracted with chloroform (3 × 100 mL). Solvent of the three partitions (*n*-hexane, chloroform and aqueous methanol) was removed in vacuo.

Denhamia obscura seeds (26.76 g) were frozen in liquid nitrogen, ground in a mortar, suspended in methanol (100 mL) and sonicated (30 min). Supernatant was decanted and solvent removed under reduced pressure to give a crude extract (2.56 g, 9.6 wt/wt%). Material was subsequently extracted with *n*-hexane (100

mL). Analysis of the extracts by GCMS and LCMS allowed for comparison of the chemical profiles of the root bark and seeds.

Denhamia obscura leaves (112.35 g) were milled, suspended in *n*-hexane (800 mL) and sonicated (45 min). The leaf extract was decanted and solvent reduced (to 200 mL) under reduced pressure. The process was repeated on the same plant sample using methanol. The *n*-hexane extract was partitioned with aqueous methanol (3 × 100 mL). Aqueous methanol was then extracted with chloroform (3 × 100 mL). The methanol extract was extracted with *n*-hexane (3 × 100 mL) and chloroform (3 × 100 mL).

Isolation of Root bark compounds: *n*-Hexane and chloroform partitions were fractionated in a Shimadzu High Performance Liquid Chromatography (HPLC) system on a Luna Silica semi-prep column (250 × 10 mm, 5 μm, 100 Å). Both samples were separated with a binary solvent system [*n*-hexane (A), ethyl acetate (B)] at a flow rate of 1 mL/min, monitored via a UV detector at 254 and 360 nm.

The fractionation of the *n*-hexane extract was achieved with mobile phase B held at 60% for 2.5 min followed by linear gradient of B from 60-95% (2.5-15 min), where it was held for 7.5 min, before a droping to 60% over 10 sec, where it was held until stopped at 25 min, with the column maintained at 50 °C, and a total of 4 purified fractions were collected (Compounds **1**, **2**, **9**, **14**).

The fractionation of the chloroform extract was obtained with mobile phase B held at 5% for 5 min followed by linear gradient of B from 5-95% (5-30 min), where it was held for 15 min, before a drop 95-5% over 5 min, where it was held until stop at 55 min, with the column oven at 40 °C, and a total of 17 purified fractions were collected (Compounds **1-13**).

Aqueous methanol partition was carried out on a Shimadzu HPLC system on a Luna C18 semi-prep column (250 × 10 mm, 5 μm, 100 Å). Both samples were separated with a binary solvent system, solvent A (acetonitrile) and solvent B (water) at a flow rate of 1 mL/min monitored with UV detector at 291 and 360 nm, and ELSD.

The fractionation of the aqueous methanol extract was obtained with mobile phase B held at 50% for 5 min followed by linear gradient of B from 50-95% (5-30 min), where it was held for 15 min, before a drop 95-5% over 0.5 min, where it was held until stop at 60 min, with the column oven at 40 °C, and a total of 6 purified fractions were collected (compounds **2**, **3**, **8**, **12**, **14**).

Isolation of compounds from leaves. The *n*-hexane extract (20 g) was fractionated over silica (385 g, Sigma-Aldrich 60 Å, 230-400 mesh, 40-63 μm particle size) with a dichloromethane (DCM)/petroleum spirit solvent mixture. The extract loaded in 10% DCM/pet. spirit (30 mL), elution started with 20% DCM/pet. spirit (300 mL), followed by 40% DCM/pet. spirit (200 mL), followed by 100% DCM, collecting 10 mL fractions. Based on GCMS and NMR screening, fractions 3-10 were combined and re-fractionated using a Shimadzu High Performance Liquid Chromatography system on a Luna Silica semi-prep column (250 × 10 mm, 5 μm, 100 Å). Sample was separated with a binary solvent system [*n*-hexane (A), ethyl acetate (B)] at a flow rate of 1 mL/min, monitored with UV detector at 243 and 316 nm and ELSD.

The fractionation of *n*-hexane extract was obtained with mobile phase B held at 20% for 5 min followed by linear gradient of B from 20-60% (5-35 min), where it was held for 3.5 min, before equilibrating until stop at 40 min, with the column oven at 45 °C, and a total of 8 fractions were collected.

Aqueous methanol partition was carried out on a Shimadzu HPLC system on a Luna C18 semi-prep column (250 × 10 mm, 5 μm, 100 Å). Sample was separated with a binary solvent system, solvent A (acetonitrile) and solvent B (water) at a flow rate of 1 mL/min, monitored with UV detector at 291 and 360 nm, and ELSD.

The fractionation of the aqueous methanol extract was obtained with mobile phase A held at 50% for 2.5 min followed by linear gradient of A from 50-95% (5-15 min), where it was held for 10 min, before equilibrating until stop at 27.5 min, with the column oven at 45 °C, and a total of 3 fractions were collected (Compounds **19**-**21**).

Denhamia obscura leaves ground material (2.5 g) was packed into SPE cartridge (Strata, 25 mL) and extracted with DCM (250 mL), solvent removed in vacuo and suspended in acetonitrile (2 mL), as per Wibowo et al.[37] Resulting solution was eluted using a Shimadzu HPLC system on a Luna Silica semi-prep column (250 × 10 mm, 5 μm, 100 Å). Sample was separated with a binary solvent system [*n*-hexane (A), ethyl acetate (B)] at a flow rate of 1 mL/min, monitored with UV detector at 243 and 316 nm. The fractionation of DCM extract was obtained with mobile phase B held at 80% for 2.5 min followed by linear gradient of B from 80-95% (2.5-20 min), where it was held for 5 min, before re-equilibration, where it was held until stop at 30 min, with the column oven at 50 °C, and a total of 5 fractions were collected. With compounds **12**, **15**-**17** present in fraction 2 and 3, **14** and **18** present in fraction 4.

Characterization

Ferruginol (**1**): 4.00 mg (0.07 wt/wt% crude extract) amorphous yellow solid; **GCMS (*m/z*)** 286.30 $[M]^{\cdot+}$. **LCMS *(m/z)*** 287.00 $[M+H]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 6.83 (s, 1H, H-14), 6.63 (s, 1H, H-11), 4.48 (s, 1H, OH-12), 3.11 (sept, *J* 7.0 Hz, 1H, H-15), 2.86 (ddd, *J* 16.5, 7.2, 2.6 Hz, 1H, H-7a), 2.77 (ddd, *J* 16.5, 10.6, 7.2 Hz, 1H, H-7b), 2.17 (dt, *J* 12.3, 3.6 Hz, 1H, H-1a), 1.85 (ddt, *J* 13.5, 7.5, 1.9 Hz, 1H, H-6a), 1.73 (dt, *J* 13.7, 3.6 Hz, 1H, H-2a), 1.66 (ddd, *J* 13.3, 6.9, 1.2 Hz, 1H, H-6b), 1.60 (dt, *J* 13.7, 3.6 Hz, 1H, H-2b), 1.47 (dtd, *J* 13.2, 3.3, 1.6 Hz, 1H, H-3a), 1.38 (td, *J* 12.3, 3.6 Hz, 1H, H-1b), 1.32 (dd, *J* 12.4, 2.3 Hz, 1H, H-5), 1.24 (d, *J* 7.0 Hz, 3H, CH_3-16), 1.22 (d, *J* 7.0 Hz, 3H, CH_3-17), 1.17 (s, 3H, CH_3-20), 0.94 (s, 3H, CH_3-19), 0.91 (s, 3H, CH_3-18). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 152.9 (C12), 148.2 (C9), 131.3 (C13), 127.3 (C8), 126.8 (C14), 111.1 (C11), 50.2 (C5), 41.5 (C3), 38.7 (C1), 38.2 (C10), 33.4 (C4), 33.1 (C19), 32.8 (C20), 29.6 (C7), 26.6 (C15), , 22.5 (C16), 22.3 (C17), 21.4 (C18), 19.2 (C2), 19.0 (C6). NMR consistent with literature.[14,38]

Maytenoquinone (**2**): 6.00 mg (0.11 wt/wt% crude extract) amorphous red solid. **UV (λ_{max}):** 230, 345 nm. **GCMS *(m/z)*** 314.30 $[M]^{\cdot+}$. **LCMS *(m/z)*** 315.05 $[M+H]^{\cdot+}$, 313.02 $[M-H]^{\cdot-}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.16 (s, 1H, OH-13),6.64 (d, *J* 1.5 Hz, 1H, H-7), 6.42 (d, *J* 1.5 Hz, 1H, H-11), 3.08 (sept, *J* 7.2 Hz, 1H, H-15), 2.52 (s, 1H, H-5), 2.04-2.01 (m, 1H, H-1), 1.78 (qt, *J* 10.4, 3.0 Hz, 1H, H-2b), 1.72 (t, *J* 3.0 Hz, 1H, H-2a), 1.51-1.47 (m, 1H, H-3a), 1.39 (d, *J* 7.0 Hz, 3H, CH_3-16), 1.33 (d, *J* 7.0 Hz, 3H, CH_3-17), 1.28 (s, 3H, CH_3-18), 1.27 (s, 3H, CH_3-19), 1.26-1.22 (m, 1H, H-3b), 1.19 (s, 3H, CH_3-20). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 199.9 (C6), 181.5 (C12), 161.6 (C9), 146.7 (C14), 140.2 (C8), 131.1 (C7),126.5 (C13), 119.9 (C11), 62.0 (C5), 42.9 (C10), 42.3 (C3), 37.4 (C1), 32.9 (C20), 26.8 (C15), 26.2 (C19), 24.5 (C4), 21.7 (C18), 20.2 (C17), 20.1 (C16), 18.4 (C2). NMR consistent with literature.[13]

Obscurol [6,13-dihydroxy-12-oxo-7,9(11),13-totaratriene] (**3**): **GCMS *(m/z)*** 316.40 $[M]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 6.95 (s, 1H, OH-13), 6.88 (s, 1H, H-7), 6.27 (s, 1H, H-11), 4.69 (br d, *J* 10.0 Hz, 1H, H-6), 3.11 (sept, *J* 7.0 Hz, 1H, H-15), 1.89 (d, *J* 10.0 Hz, 1H, H-5), 1.68-1.62 (m, 1H, H-2), 1.36 (d, *J* 7.0 Hz, 3H, CH_3-16), 1.33 (d, *J* 7.0 Hz, 3H, CH_3-17), 1.21 (s, 3H, CH_3-20), 1.18 (s, 3H, CH_3-19), 1.13 (s, 3H, CH_3-18). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 161.6 (C14), 143.6 (C7), 135.8 (C8), 126.2 (C13), 117.4 (C11), 69.9 (C6), 37.6 (C5), 36.2 (C20), 26.6 (C15), 22.6 (C19), 23.3 (C18), 20.4 (C16), 20.2 (C17), 18.6 (C2). NMR consistent with literature.[12]

Dispermol (**4**): amorphous yellow solid. **GCMS *(m/z)*** 316.10 $[M]^{\cdot+}$. **LCMS *(m/z)*** 315.04 $[M-H]^{\cdot-}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 6.77 (s, 1H, H-11), 3.77 (s, 3H, 12-OCH_3), 3.40-3.27 (m, 1H, H-15), 2.88 (dd, *J* 16.6, 6.7 Hz, 1H, H-7b), 2.70 (td, *J* 11.0, 5.4 Hz, 1H, H-7a), 2.17 (dt, *J* 13.5, 4.0 Hz, 1H, H-1a), 1.88 (dd, *J* 13.1, 3.3 Hz, 1H, H-6a), 1.76 (dt, *J* 13.4, 3.3 Hz, 1H, H-2a), 1.63-1.60 (m, 1H, H-6b), 1.62-1.58 (m, 1H, H-2b), 1.47-1.43 (m, 1H, H-3a),

1.38-1.36 (m, 1H, H-1b), 1.34 (d, *J* 7.0 Hz, 3H, CH_3-16), 1.32 (d, *J* 7.0 Hz, 3H, CH_3-17), 1.24-1.22 (m, 1H, H-3b), 1.17 (s, 3H, CH_3-20), 0.94 (s, 3H, CH_3-19), 0.89 (s, 3H, CH_3-18). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 147.2 (C13), 146.4 (C12), 143.6 (C9), 140.9 (C14), 109.8 (C11), 61.2 (12-OCH_3), 49.8 (C5), 41.4 (C3), 39.3 (C1), 38.7 (C4), 33.3 (C18), 33.2 (C10), 33.0 (C19), 27.3 (C15), 24.8 (C20), 21.4 (C19), 20.4 (C16, C17), 20.2 (C6), 18.5 (C2). NMR consistent with literature.[10]

12-Hydroxytotarol (**5**): amorphous yellow solid. **GCMS *(m/z)*** 284.30 $[M\text{-}OH]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 6.66 (s, 1H, H-11), 5.17 (s, 1H, OH-12), 4.70 (s, 1H, OH-13), 3.26 (sept, *J* 7.0 Hz, 1H, H-15), 2.85 (dd, *J* 16.7, 6.6 Hz, 1H, H-7eq), 2.67 (dt, *J* 17.1, 8.5 Hz, 1H, H-7a), 2.12 (ddd, *J* 12.4, 6.8, 1.6 Hz, 1H, H-1a), 1.89 (ddt, *J* 13.4, 7.9, 1.9 Hz, 1H, H-6_{eq}), 1.71 (qt, *J* 13.8, 3.5 Hz, 1H, H-2_{ax}), 1.63 (ddd, *J* 13.1, 6.6, 1.4 Hz, 1H, H-6_{ax}), 1.57 (dt, *J* 13.7, 3.3 Hz, 1H, H-2_{eq}), 1.46 (dtd, *J* 13.1, 3.3, 1.4 Hz, 1H, H-3_{ax}), 1.36 (d, *J* 7.0 Hz, 3H, CH_3-16), 1.34 (d, *J* 7.0 Hz, 3H, CH_3-17), 1.35-1.28 (m, 1H, H-1b), 1.25 (dq, *J* 12.7, 1.9 Hz, 1H, H-5), 1.21 (dt, *J* 13.1, 4.2 Hz, 1H, H-3_{eq}), 1.16 (s, 3H, CH_3-20), 0.94 (s, 3H, CH_3-19), 0.90 (s, 3H, CH_3-18). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 109.7 (C11), 49.7 (C5), 41.5 (C3), 39.6 (C1), 33.1 (C19), 28.1 (C7), 27.4 (C15), 25.0 (C20), 21.5 (C18), 20.3 (C16), 20.2 (C17), 19.4 (C2), 19.3 (C6). NMR consistent with literature.[12]

Sugiol (**6**): 2.00 mg (0.04 wt/wt% crude extract) amorphous red solid. **GCMS *(m/z)*** 300.30 $[M]^{\cdot+}$. **LCMS *(m/z)*** 301.00 $[M+H]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.90 (s, 1H, H-14),6.69 (s, 1H, H-11), 3.23 (sept, *J* 7.3 Hz, 1H, H-15), 2.66-2.63 (m, 1H, H-6a), 2.56-2.52 (m, 1H, H-6b), 2.19-2.17 (m, 1H, H-1a), 1.85 (dd, *J* 14.0, 4.0 Hz, 1H, H-5), 1.78-1.75 (m, 1H, H-2a), 1.67-1.64 (m, 1H, H-2b), 1.53-1.49 (m, 1H, H-1a), 1.52-1.49 (m, 1H, H-3a), 1.27-1.25 (m, 1H, H-3b), 1.24 (d, *J* 7.3 Hz, CH_3-16), 1.22 (d, *J* 7.3 Hz, CH_3-17), 1.21 (s, 3H, CH_3-20), 0.98 (s, 3H, CH_3-19), 0.92 (s, 3H, CH_3-18). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 198.6 (C7), 157.9 (C12), 156.3 (C9), 131.7 (C13), 126.6 (C14), 125.7 (C8), 109.7 (C11), 49.5 (C5), 41.5 (C3), 36.9 (C1), 33.5 (C4), 33.0 (C18), 26.9 (C15), 25.8 (C16), 23.0 (C20), 22.4 (C6), 22.3 (C17), 22.30 (C3), 21.3 (C19), 18.7 (C2). NMR consistent with literature.[14,39]

13-Methoxy-sempervir-6-ene (**7**): amorphous yellow solid. **UV (λ_{max}):** 224, 280 nm. **LCMS *(m/z)*** 301.16 $[M+H]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 6.84 (dd, *J* 10.0, 3.2 Hz, 1H, H-7), 6.71 (s, 1H, H-11), 6.61 (s, 1H, H-14), 5.88 (dd, *J* 10.0, 2.9 Hz, 1H, H-6), 3.75 (s, 3H, OCH_3-21), 3.06 (sept, *J* 6.8 Hz, 1H, H-15), 2.52 (dt, *J* 13.4, 3.3 Hz, 1H, H-1b), 2.05-2.01 (m, 1H, H-5), 1.87 (q, *J* 11.7 Hz, 1H, H-2b), 1.70 (d, *J* 13.7 Hz, 1H, H-1a), 1.68 (d, *J* 15.3 Hz, 1H, H-2a), 1.45 (dtd, *J* 12.7, 3.1, 1.5 Hz, 1H, H-3b), 1.34 (d, *J* 4.8 Hz, 3H, CH_3-16), 1.32 (d, *J* 4.8 Hz, 3H, CH_3-17), 1.19-1.15 (m, 1H, H-3a), 1.04 (s, 3H, CH_3-19), 1.01 (s, 3H, CH_3-18), 0.94 (s, 3H, CH_3-20). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 147.3 (C13), 142.5 (C9), 141.2 (C8), 131.0 (C14), 123.9 (C6), 122.8 (C7), 114.0 (C11), 61.4 (C21), 49.8 (C5), 41.5 (C3), 38.4 (C1), 33.1 (C20), 27.2 (C15), 22.6 (C19), 21.4 (C17), 21.3 (C16), 20.8 (C18), 17.7 (C2).

Pristimerin (**8**): 6.97 mg (0.12 wt/wt% crude extract) amorphous red solid, **UV (λ_{max}):** 247, 438 nm. **GCMS *(m/z)*** 464.40 $[M]^{\cdot+}$. **LCMS *(m/z)*** 465.26 $[M+H]^{\cdot+}$, 463.14 $[M\text{-}H]^{\cdot-}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.02 (dd, *J* 7.1, 1.5 Hz, 1H, H-6), 6.53 (d, *J* 1.5 Hz, 1H, H-1), 6.35 (d, *J* 7.1 Hz, 1H, H-7), 3.55 (s, 3H, CH_3-24), 2.42 (br d, *J* 15.8 Hz, 1H, H-19a), 2.21 (s, 3H, CH_3-23), 2.20 (d, *J* 4.0 Hz, 1H, H-21a), 2.17 (d, *J* 4.0 Hz, 1H, H-11a), 2.05 (td, *J* 14.2, 4.1 Hz, 1H, H-22a), 1.90 (dd, *J* 14.1, 6.1 Hz, 1H, H-16b), 1.84 (td, *J* 13.0, 4.0 Hz, 1H, H-11b), 1.81-1.77 (m, 1H, H-12a), 1.72 (d, *J* 8.0, 1H, H-19b), 1.68 (d, *J* 13.0 Hz, 1H, H-12b), 1.69-1.66 (m, 1H, H-15a), 1.59 (d, *J* 8.0 Hz, 1H, H-18), 1.57 (td, *J* 14.1, 6.1 Hz, 1H, H-15b), 1.49 (ddd, *J* 14.1, 6.1, 1.9 Hz, 1H, H-16a), 1.45 (s, 3H, CH_3-25), 1.38 (t, *J* 4.0 Hz, 1H, H-21b), 1.26 (s, 3H, CH_3-26), 1.18 (s, 3H, CH_3-30), 1.10 (s, 3H, CH_3-28), 0.96 (ddd, *J* 14.0, 4.0, 2.0 Hz, 1H, H-22b), 0.53 (s, 3H, CH_3-27). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 178.7 (C29), 178.2 (C2), 170.2 (C8), 164.8 (C10), 146.0 (C3), 134.2 (C6), 127.4 (C5), 119.5 (C1), 118.1 (C7), 117.3 (C4), 51.6 (C24), 45.1 (C14), 44.3 (C18), 43.0 (C9), 40.4 (C20), 39.4 (C13), 38.3 (C25), 36.3 (C16), 34.8 (C22), 33.6 (C11), 32.7 (C30), 31.6 (C28), 30.9 (C19), 30.5 (C17), 29.9 (C21), 29.7 (C12), 28.6 (C15), 21.6 (C26), 18.3 (C27), 10.2 (C23).NMR consistent with literature.[40]

Celastrol (**9**): 4.97 mg (0.09 wt/wt% crude extract) amorphous red solid. **UV (λ_{max}):** 247, 438 nm. **LCMS *(m/z)*** 451.16 $[M+H]^{\cdot+}$, 449.14 $[M-H]^{\cdot-}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.06 (dd, *J* 7.2, 1.4 Hz, 1H, H-1), 6.50 (d, *J* 1.4 Hz, 1H, H-6), 6.33 (d, *J* 7.2 Hz, 1H, H-7), 2.49 (d, *J* 15.0 Hz, 1H, H-19b), 2.24 (d, *J* 15.0 Hz, 1H, H-21b), 2.21 (s, 3H, CH_3-23), 2.10 (tt, *J* 13.8, 4.2 Hz, 2H, H-11a), 1.85 (dd, *J* 13.8, 4.2 Hz, 1H, H-12b), 1.82-1.78 (m, 1H, H-19a), 1.59-1.57 (m, 1H, H-18), 1.47 (dd, *J* 15.0, 3.7, 2H, H-16), 1.43 (s, 3H, CH_3-25), 1.36 (td, *J* 14.0, 4.6 Hz, 1H, H-21a), 1.27 (s, 3H, CH_3-28), 1.25 (s, 3H, CH_3-26), 1.09 (s, 3H, CH_3-24), 0.95 (d, *J* 15.0 Hz, 1H, H-22b), 0.59 (s, 3H, CH_3-27). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 135.2 (C1), 120.2 (C6), 118.3 (C7), 44.1 (C18), 38.4 (C25), 36.5 (C16), 34.0 (C11), 32.4 (C28), 31.5 (C24), 30.9 (C19), 29.2 (C12), 21.3 (C26), 18.9 (C27), 10.1 (C23). NMR consistent with literature.[41,42]

Tingenone (**10**): 2.00 mg (0.04 wt/wt% crude extract) amorphous red solid. **UV (λ_{max}):** 318 nm. **LCMS *(m/z)*** 419.08 $[M-H]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.02 (d, *J* 1.4 Hz, 1H, H-6), 6.95 (s, 1H, 3-OH), 6.53 (d, *J* 1.4 Hz, 1H, H-1), 6.35 (d, *J* 7.2 Hz, 1H, H-7), 2.54 (d, *J* 4.7 Hz, 1H, H-20), 2.26 (d, *J* 3.7 Hz, 1H, H-11b), 2.24 (s, 3H, CH_3-23), 1.85 (dd, *J* 8.9, 3.0 Hz, 1H, H-22a), 1.84 (d, *J* 2.8 Hz, 1H, H-12a), 1.82 (s, 1H, H-15b), 1.77 (td, *J* 8.0, 2.9 Hz, 2H, H-19), 1.67 (s, 1H, H-18), 1.66 (s, 1H, H-15a), 1.49 (s, 3H, CH_3-25), 1.46 (s, 1H, CH_2-16a), 1.36 (s, 3H, CH_3-26), 1.01 (s, 3H, CH_3-24), 1.00 (d, *J* 4.7 Hz, 3H, CH_3-28), 0.97 (s, 3H, CH_3-27). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 212.9 (C21), 178.4 (C2), 168.8 (C8), 165.0 (C10), 146.7 (C3), 134.3 (C1), 133.8 (C6), 128.2 (C5), 119.7 (C1), 117.6 (C4), 43.0 (C18), 41.4 (C20), 34.0 (C11), 28.1 (C24), 27.9 (C15), 27.1 (C28), 26.9 (C28), 26.8 (C27), 26.8 (C27), 21.9 (C23), 21.7 (C23), 20.4 (C25), 18.0 (C25). NMR consistent with literature.[43]

Iguesterin (**11**): 0.97 mg (0.02 wt/wt% crude extract) amorphous red solid. **UV (λ_{max}):** 318 nm. **LCMS *(m/z)*** 405.06 $[M+H]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 7.03 (dd, *J* 7.2, 1.4 Hz, 1H, H-1), 6.53 (d, *J* 1.4 Hz, 1H, OH-3), 6.35 (d, *J* 7.2 Hz, 1H, H-6), 5.24 (d, *J* 6.0 Hz, 1H, H-7), 1.77 (d, *J* 3.0 Hz, 3H, CH_3-28), 1.47 (s, 3H, CH_3-25), 1.44 (s, 3H, CH_3-24), 0.94 (s, 3H, CH_3-26), 0.51 (s, 3H, CH_3-23). **^{13}C NMR (125 MHz, $CDCl_3$)** δ_C 134.1 (C1), 119.4 (C3), 118.0 (C6), 29.6 (C28), 41.6 (C25), 38.0 (C24), 33.1 (C26), 22.2 (C23). NMR consistent with literature.[43]

Friedelin (**12**): colorless crystals, [a] -12° (c = 0.4, $CHCl_3$), lit., [a] -13.5° (c = 0.1, $CHCl_3$). **M.p.** 263.4-265.3 °C.[44] **LRMS ESI (*m/z*)** 427.28 $[M+H]^{\cdot+}$. **GCMS (*m/z*)** 426.16 $[M]^{\cdot+}$. **^{1}H (500 MHz, $CDCl_3$)** δ_H 2.39 (ddd, *J* 14.0, 5.1, 2.0 Hz, 1H, H-2a), 2.30 (ddd, *J* 14.0, 7.2, 1.2 Hz, 1H, H-2b), 2.25 (q, *J* 7.1 Hz, 1H, H-4), 1.97 (ddt, *J* 13.0, 7.1, 2.0 Hz, 1H, H-1a), 1.75 (dt, *J* 14.0, 2.9 Hz, 1H, H-6a), 1.68 (dd, *J* 13.0, 5.1 Hz, 1H, H-1b), 1.62-1.58 (m, 1H, H-16a), 1.57-1.55 (m, 1H, H-18), 1.54 (brd s, 1H, H-10), 1.53 (s, 2H, H-16b), 1.51-1.49 (m, 1H, H-7a), 1.48 (br s, 2H, H-15a, H-22a), 1.46 (dd, *J* 8.4, 4.4 Hz, 1H, H-11a), 1.39 (s, 1H, H-8), 1.37 (q, *J* 2.7 Hz, 1H, H-7a), 1.35 (d, *J* 3.7 Hz, 1H, H-19a), 1.34 (s, 2H, H-12), 1.29-1.26 (m, 1H, H-6b), 1.28-1.25 (m, 1H, 11b), 1.25 (s, 1H, H-17), 1.21 (dd, *J* 14.0, 5.9 Hz, 1H, H-19b), 1.18 (s, 3H, H-28), 1.05 (s, 3H, H-27), 1.01 (s, 3H, H-26), 1.00 (s, 3H, H-29), 0.95 (s, 3H, H-30), 0.94 (s, 2H, H-21), 0.88 (d, *J* 6.8 Hz, 3H, H-23), 0.87 (s, 3H, H-25), 0.72 (s, 3H, H-24). **^{13}C (500 MHz, $CDCl_3$)** δ_C 213.2 (C3), 59.5 (C10), 58.2 (C4), 53.1 (C8), 42.8 (C18), 42.1 (C5), 41.5 (C2), 41.3 (C6), 39.7 (C14), 39.2 (C22), 38.3 (C13), 37.4 (C9), 36.0 (C16), 35.6 (C11), 35.3 (C19), 35.0 (C29), 32.7 (C21), 32.4 (C15), 32.1 (C28), 31.8 (C30), 30.5 (C12), 29.9 (C17), 28.2 (20), 22.3 (C1), 20.2 (C26), 18.6 (C27), 18.2 (C7), 17.9 (C25), 14.6 (C24), 6.8 (C23). NMR consistent with literature.[27,28]

Multiflorenol (**13**): **GCMS *(m/z)*** 426.5 $[M]^{\cdot+}$. **^{1}H NMR (500 MHz, $CDCl_3$)** δ_H 5.45 (br s, 1H, H-7), 3.26 (dd, *J* 11.3, 4.0 Hz, 1H, H-3), 1.08 (s, 3H, H-25), 1.07 (s, 3H, H-26), 1.05 (s, 3H, H-27), 0.97 (s, 3H, H-29), 0.96 (s, 3H, H-30), 0.88 (s, 3H, H-24), 0.86 (s, 3H, H-23), 0.75 (s, 3H, H-28). NMR consistent with literature.[45]

Glutinol (**14**): **GCMS *(m/z)*** 426.50 $[M]^{\cdot+}$. **^{1}H (500 MHz, $CDCl_3$)** δ_H 5.63 (d, *J* 5.7 Hz, 1H, H-6), 3.47 (t, *J* 3.0 Hz, 1H, H-3), 1.16 (s, 3H, CH_3-28), 1.14 (s, 3H, H-24), 1.07 (s, 3H, CH_3-27), 1.04 (s, 3H, CH_3-23), 0.99 (s, 3H, CH_3-26), 0.95 (s, 3H, CH_3-30), 0.94 (s, 3H, CH_3-29), 0.85 (s, 3H, CH_3-25). **^{13}C (500 MHz, $CDCl_3$)** δ_C 122.0 (C6), 76.3 (C3), 34.8 (C29), 32.1 (C28), 28.9 (C23), 25.6 (C24), 19.2 (C-27), 18.7 (C26), 16.1 (C25). NMR consistent with literature.[46,47]

Lupen-3-one (**15**), amorphous colorless solid. **GCMS (*m/z*)** 424.40 $[M]^{\cdot+}$. **^{1}H (500 MHz, $CDCl_3$)** δ_H 4.63 (br s, 1H, H-29b), 4.50 (br s, 1H, H-29a), 2.43-2.42 (m, 2H, H-2), 2.35-2.33 (m, 1H, H-19), 1.62 (s, 3H, H-30), 0.97 (s, 3H, H-23), 0.97 (s, 3H, H-26), 0.87 (s, 3H, H-27), 0.76 (s, 3H, H-25), 0.69 (s, 3H, H-28). **^{13}C (500 MHz, $CDCl_3$)** δ_C 150.6 (20), 109.3 (29), 49.7 (9), 48.1 (18), 47.8 (19), 47.2 (4), 43.2 (17), 42.9 (14), 40.7 (8), 40.5 (22), 39.6 (1), 38.2 (13), 36.8 (10), 35.5 (16), 34.2 (2), 33.5 (7), 29.9 (21), 27.4 (15), 26.8 (23), 25.2 (12), 21.4 (11), 20.8 (24), 19.6 (6), 19.2 (30), 17.9 (28), 15.7 (26), 14.4 (27). ^{1}H and ^{13}C NMR acquired from a mixture of compounds (**1**-**3**). Data consistent with literature.[27,28]

Germanicone (**16**), amorphous colorless solid, **GCMS (*m/z*)** 424.40 $[M]^{\cdot+}$. **^{1}H (500 MHz, $CDCl_3$)** δ_H 4.88 (br s, 1H, H-19), 1.10 (s, 3H, H-26), 1.07 (s, 3H, H-23), 1.03 (s, 3H, H-24), 1.02 (s, 3H, H-28), 0.96 (s, 3H, H-25), 0.95 (s, 6H, H-29, H-30), 0.75 (s, 3H, H-27). **^{13}C (500 MHz, $CDCl_3$)** δ_C 218.2 (3), 142.5 (18), 129.9 (19), 54.9 (5), 50.5 (9), 47.3 (4), 43.4 (14), 40.6 (8), 39.9 (1), 38.9 (13), 37.6 (22), 37.4 (16), 36.9 (10), 34.3 (17), 34.1 (2), 33.8 (7), 33.3 (21), 32.4 (20), 31.3 (29), 29.2 (30), 27.5 (15), 26.9 (23), 26.2 (12), 25.3 (28), 21.7 (11), 20.9 (24), 19.7 (6), 16.6 (25), 15.7 (26), 14.5 (27). ^{1}H and ^{13}C NMR acquired from a mixture of compounds **1**-**3**. Data consistent with literature.[29]

β-Amyrone (**17**), amorphous colorless solid. **GCMS (*m/z*)** 424.40 $[M]^{\cdot+}$. **^{1}H (500 MHz, $CDCl_3$)** δ_H 5.20 (br s, 1H, H-12), 1.10 (s, 3H, H-23), 1.08 (s, 3H, H-25), 1.05 (s, 3H, H-24), 1.03 (s, 3H, H-26), 0.88 (s, 6H, H-29, H-30), 0.84 (s, 6H, H-27, H-28). **^{13}C (500 MHz, $CDCl_3$)** δ_C 217.9 (3), 145.4 (13), 121.5 (12), 55.4 (5), 47.5 (4), 46.9 (9), 46.8 (19), 41.8 (14), 39.8 (8), 39.3 (1), 37.0 (22), 36.7 (10), 34.7 (21), 33.4 (29), 32.6 (17), 32.1 (7), 31.0 (20), 28.4 (28), 26.9 (16), 26.5 (30), 26.1 (15), 25.9 (27), 23.7 (30), 23.6 (11), 21.5 (24), 19.6 (6), 16.8 (26), 15.2 (25). ^{1}H and ^{13}C NMR acquired from a mixture of compounds (**1**-**3**). Data consistent with literature.[30]

Lupeol (**18**): **GCMS *(m/z)*** 426.70 $[M]^{\cdot+}$. **^{1}H (500 MHz, $CDCl_3$)** δ_H 4.67 (br s, 1H, H-29a), 4.57 (br s, 1H, H-29b), 1.68 (s, 3H, CH_3-30), 1.03 (s, 3H, CH_3-26), 0.96 (s, 3H, CH_3-23), 0.93 (s, 3H, CH_3-27), 0.84 (s, 3H, CH_3-25) 0.79 (s, 3H, CH_3-28). **^{13}C (500 MHz, $CDCl_3$)** δ_C 106.1 (29), 19.1 (30), 15.7 (27), 15.7 (26). ^{1}H and ^{13}C NMR as a mixture with Glutinol (**5**). NMR data consistent with literature.[48]

Biological testing

The antibacterial and antifungal activities were evaluated in a microtiter broth assay based on Clinical Laboratory Standards Institute (CLSI) Standard Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically[19] and Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts.[20] The extracts were screened for activity against bacterial reference strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25668), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615) and yeast reference strain *Candida albicans* (ATCC 90029).

The crude extracts were assessed in 96 well microtiter plates in a final volume of 150 μL at a final concentration of 20 mg/mL in 2% DMSO. Growth controls included microbe in growth media, microbe in growth media with 2% DMSO solution. Growth inhibition control included microbe in growth media with 0.1% povidone-iodine (w/v, Betadine, Sanofi).

Escherichia coli and *P. aeruginosa* were inoculated in cation adjusted Mueller Hinton broth, *S. aureus* and *S. pyogenes* in Todd Hewitt Broth with 2% Yeast Extract at a final concentration of 5×10^5 CFU/mL. *Candida albicans* was inoculated in Sabouraud broth at a final concentration of 1.25×10^5 CFU/mL. Microbe growth assessed by optical density at 595 nm using a plate reader (Victor X2, Perkin Elmer). The increase in OD at 20 h for bacteria, and 24 h for yeast compared to the initial OD reading was used to determine the percentage growth inhibition compared to the growth control. Each extract and control was tested in triplicate in at least three independent experiments.[19,20]

Acknowledgements

The authors acknowledge Australian First Nation peoples as the owners of the lands where these samples were collected and holders of Traditional Knowledge of the use and applications of *Denhamia obscura*. The Hewitt family are acknowledged for their contribution in supporting the project on their country. The authors also acknowledge the financial contribution to this research from the Cooperative Research Centre for Developing Northern Australia through the project "Enabling a Traditional Medicinal Plants Agribusiness" (HT.2.1718007).

Supplementary Material

General Experimental parameters, details of the crystal structure and NMR spectra for obscurol (**3**) and 13-methoxy-sempervir-6-ene (**7**) can be found in the SI information.

References

1. McKenna, M. J.; Simmons, M. P.; Bacon, C. D.; Lombardi, J. A. *Syst. Bot.* **2011**, *36*, 922. https://doi.org/10.1600/036364411X604930
2. Simmons, M. P.; Cappa, J. J.; Archer, R. H.; Ford, A. J.; Eichstedt, D.; Clevinger, C. C. *Mol. Phylogenet. Evol.* **2008**, *48*, 745. https://doi.org/10.1016/j.ympev.2008.04.039
3. Gordon, S.; Wibowo, M.; Wang, Q.; Holst, J.; Davis, R. A. *Magn. Reson. Chem.* **2018**, *57*, 101. https://doi.org/10.1002/mrc.4801
4. Levrier, C.; Sadowski, M. C.; Nelson, C. C.; Healy, P. C.; Davis, R. A. *J. Nat. Prod.* **2015**, *78*, 111. https://doi.org/10.1021/np500740f
5. Wibowo, M.; Wang, Q.; Holst, J.; White, J. M.; Hofmann, A.; Davis, R. A. *Asian J. Org. Chem.* **2016**, *5*, 1461. https://doi.org/10.1002/ajoc.201600462
6. Appetiti, E. In *Handbook of Medicinal Plants*; Yaniv, Z., Bachrach, U., Eds. 2005.
7. Puruntatameri, J.; Puruntatameri, R.; Pangiraminni, A.; Burak, L.; Tipuamantymirri, C.; Tipakalippa, M.; Puruntatameri, J.; Puruntatameri, P.; Pupangamirri, J. B.; Kerinaiua, R.; Tipiloura, D.; Orsto, M.-M.; Kantilla, B.; Kurrupuwu, M.; Puruntatameri, P. F.; Puruntatameri, T. D.; Puruntatameri, L.; Kantilla, K.; Wilson, J.; Cusack, J.; Jackson, D.; Wightman, G. *Tiwi Plants and Animals: aboriginal flora and fauna knowledge from Bathurst and Melville Islands, northern Australia*; Tiwi Land Council, 2001.
8. Hyland, B. P. M.; Whiffin, T.; Zich, F. A.; Duffy, S.; Gray, B.; Elick, R.; Venter, F.; Christophel, D. In *Australian Tropical Rainforest Plants*; CSIRO: 2010;
9. Ardiles, A. E.; Gonzalez-Rodriguez, A.; Nunez, M. J.; Perestelo, N. R.; Pardo, V.; Jimenez, I. A.; Valverde, A. M.; Bazzocchi, I. L. *Phytochemistry* **2012**, *84*, 116. https://doi.org/10.1016/j.phytochem.2012.07.025
10. Martín, J. D. *Tetrahedron* **1973**, *29*, 2553. https://doi.org/10.1016/0040-4020(73)80172-3

11. Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1994**, *57*, 1675. https://pubs.acs.org/doi/pdfplus/10.1021/np50114a009
12. Cambie, R. C.; Higgs, P. I.; Read, C. M.; Rutledge, P. S.; Ryan, G. R.; Woodgate, P. D. *Aust. J. Chem.* **1990**, *43*, 681.

13. Tada, M.; Kurabe, J.; Yasue, H.; Ikuta, T. *Chem. Pharm. Bull.* **2008**, *56*, 287. https://doi.org/10.1248/cpb.56.287
14. Marcos, I. S.; Beneitez, A.; Moro, R. F.; Basabe, P.; Diez, D.; Urones, J. G. *Tetrahedron* **2010**, *66*, 7773. https://doi.org/10.1016/j.tet.2010.07.066
15. Rogachev, V.; Lohl, T.; Markert, T.; Metz, P. *Arkivoc* **2012**, *iii*, 172. http://dx.doi.org/10.3998/ark.5550190.0013.313
16. Cordova-Guerrero, I.; Andres, L. S.; Leal-Orozco, A.; Padron, J. M.; Cornejo-Bravo, J. M.; Leon, F. *Tetrahedron Lett.* **2013**, *54*, 4479. http://dx.doi.org/10.1016/j.tellet.2013.06.048
17. Matsumoto, T.; Usui, S.; Fukui, K. *Chem. Lett.* **1976**, *5*, 241.

18. Zaher, A. M.; Lin, J.; Arai, M. *Nat. Prod. Commun.* **2020**, *15*, 1. https://doi.org/10.1177/1934578X20915298
19. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Ninth Edition. CLSI standard M07. Wayne, P. C. a. L. S. I.

20. CLSI. Methods for Dilution Antifungal Susceptibility Testing of Yeasts. Fourth edition. CLSI standard M27. Wayne, P. C. a. L. S. I.

21. Tada, M.; Kurabe, J.; Yoshida, T.; Ohkanda, T.; Matsumoto, Y. *Chem. Pharm. Bull.* **2010**, *58*, 818.

22. Corey, E. J.; Ursprung, J. J. *J. Am. Chem. Soc.* **1955**, *77*, 3667. https://doi.org/10.1021/ja01618a082
23. Courtney, J. L.; Gascoigne, R. M.; Szumer, A. Z. *J. Chem. Soc.* **1956**, 2119. https://doi.org/10.1039/JR9560002119
24. Djerassi, C.; Klyne, W. *J. Chem. Soc.* **1962**, 4929.

25. Jiang, R.-W.; Ma, S.-C.; He, Z.-D.; Huang, X.-S.; But, P. P.-H.; Wang, H.; Chan, S.-P.; Ooi, V. E.-C.; Xu, H.-X.; Mak, T. C. W. *Biorg. Med. Chem.* **2002**, *10*, 2161. https://doi.org/10.1016/S0968-0896(02)00072-X
26. Sousa, G. F.; Duarte, L. P.; Alcantara, A. F. C.; Silva, G. D. F.; Vieira-Filho, S. A.; Silva, R. R.; Olivaira, D. M.; Takahashi, J. A. *Molecules* **2012**, *17*, 13439.

27. Puapairoj, P.; Naengchomnong, W.; Kijjoa, A.; Pinto, M. M.; Pedro, M.; Nascimento, M. S. J.; Silva, A. M. S.; Herz, W. *Planta Med.* **2005**, *71*, 208. https://doi.org/10.1055/s-2005-837818
28. Çulhaoğlu, B.; Hatipoğlu, S. D.; Dönmez, A. A.; Topçu, G. *Med. Chem. Res.* **2015**, *24*, 3831. https://doi.org/10.1007/s00044-015-1424-7
29. Nakane, T.; Maeda, Y.; Ebihara, H.; Arai, Y.; Masuda, K.; Takano, A.; Ageta, H.; Shiojima, K.; Cai, S.-Q.;

Abdel-Halim, O. B. *Chem. Pharm. Bull.* **2002**, *50*, 1273.
https://doi.org/10.1248/cpb.50.1273
30. Akihisa, T.; Yamamoto, K.; Tamura, T.; Kimura, Y.; Iida, T.; Nambara, T.; Chang, F. C. *Chem. Pharm. Bull.* **1992**, *40*, 789.
https://doi.org/10.1248/cpb.40.789
31. Breen, G. J. W.; Ritchie, E.; Sidwell, W. T. L.; Taylor, W. C. *Aust. J. Chem.* **1966**, *19*, 455.

32. Chen, K.-S.; Chang, F.-R.; Chia, Y.-C.; Wu, T.-S.; Wu, Y.-C. *J. Chin. Chem. Soc. (Taipei, Taiwan)* **1998**, *45*, 103.

33. Thimmappa, R.; Geisler, K.; Louveau, T.; O'Maille, P.; Osbourn, A. *Annu. Rev. Plant Biol.* **2014**, *65*, 225.
https://doi.org/10.1146/annurev-arplant-050312-120229
34. Silva, F. C.; de Oliveira, M. L. G.; Rodrigues, V. G.; Carvalho, S. M.; Duarte, L. P.; Silva, G. D. F.; Miranda, R. R. S.; Figueiredo, R. C.; Moraes, J. C.; Filho, S. A. V. *Chem. Nat. Compd.* **2013**, *49*, 571.

35. Souza-Moreira, T. M.; Alves, T. B.; Pinheiro, K. A.; Felippe, L. G.; De Lima, G. M. A.; Watanabe, T. F.; Barbosa, C. C.; Santos, V. A. F. F. M.; Lopes, N. P.; Valentini, S. R.; Guido, R. V. C.; Furlan, M.; Zaneli, C. F. *Sci. Rep.* **2016**, *6*, 36858.
https://doi.org/10.1038/srep36858
36. Anderson, E. *Plants of central Queensland: identification and uses of native and introduced species*; CSIRO Publishing, 2016.

37. Wibowo, M.; Forster, P. I.; Guymer, G. P.; Hofmann, A.; Davis, R. A. *Molecules* **2019**, *24*, 859.

38. Tao, Z.; Robb, K. A.; Zhao, K.; Denmark, S. E. *J. Am. Chem. Soc.* **2018**, *140*, 3569.
https://doi.org/10.1021/jacs.8b01660
39. Yang, J.; Peng, C.-Z.; Jiang, J.; Huang, G.; Liu, Y.; Chen, Y.-G. *Chem. Nat. Compd.* **2016**, *52*, 142.
https://doi.org/10.1007/s10600-016-1573-z
40. Espindola, L. S.; Dusi, R. G.; Demarque, D. P.; Braz-Filho, R.; Yan, P.; Bokesch, H. R.; Gustafson, K. R.; Beutler, J. A. *Molecules* **2018**, *23*, 1494.
https://doi.org/10.3390/molecules23061494
41. Kutney, J. P.; Hewitt, G. M.; Kurihara, T.; Salisbury, P. J.; Sindelar, R. D.; Stuart, K. L.; Townsley, P. M.; Chalmers, W. T.; Jacoli, G. G. *Can. J. Chem.* **1981**, *59*, 2677.

42. Zhang, D.; Chen, Z.; Hu, C.; Yan, S.; Li, Z.; Lian, B.; Xu, Y.; Ding, R.; Zeng, Z.; Zhang, X.-k.; Su, Y. *Chem. Commun.* **2018**, *54*, 12871.
https://doi.org/10.1039/C8CC06140H
43. González, A. G.; López, I.; Ferro, E. A.; Ravelo, A. G.; Gutiérrez, J.; Aguilar, M. A. *Biochem. Syst. Ecol.* **1986**, *14*, 479.
https://doi.org/10.1016/0305-1978(86)90005-0
44. Kuo, Y.-H.; Kuo, L.-M. Y. *Phytochemistry* **1997**, *44*, 1275.
http://dx.doi.org/10.1016/s0031-9422(96)00719-4
45. Kushiro, T.; Shibuya, M.; Masuda, K.; Ebizuka, Y. *Tetrahedron Lett.* **2000**, *41*, 7705.

46. Yu, C.-X.; Yu, Y.-F.; Zhao, Y.; Wu, P.-Q.; Hu, Q.-L.; Li, B.; Su, P.-J.; Hu, F.-D.; Zhang, Z.-X.; Fei, D.-Q. *Chem. Nat. Compd.* **2020**, *56*, 370. https://doi.org/10.1007/s10600-020-03036-9
47. Ali, H.; Ullah, K.; Siddiqui, H.; Iqbal, S.; Atia-tul-Wahad; Goren, N.; Ayatollahi, S. A.; Atta-ur-Rahman; Choudhary, M. I. *Bioorg. Chem.* **2020**, *96*, 103526. https://doi.org/10.1016/j.bioorg.2019.103526
48. Wang, H.; Cong, W.-L.; Fu, Z.-L.; Chen, D.-F.; Wang, Q. *Nat. Prod. Res.* **2017**, *31*, 2312.