**The formation of sesquiterpenoid presilphiperfolane and cameroonane metabolites in the Bcbot4 null mutant of Botrytis cinerea†**

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**Botrytis cinerea** is a polyphagous fungal parasite which causes serious damage to more than 200 plant species and consequent economic losses for commercial crops. This pathogen produces two families of phytotoxins, the botryanes and botcinins, which are involved in the infection mechanism. The *B. cinerea* genome has provided a complete picture of the genes involved in the biosynthesis of its secondary metabolites. The botrydial biosynthetic gene cluster has been identified. This cluster consists of seven genes, where the genes *BcBOT1, BcBOT3* and *BcBOT4* encode three mono-oxygenases. A study of the *Bcbot4Δ* null mutant revealed that this mono-oxygenase was involved in the hydroxylation at C-4 of the probeotane skeleton (C-11 of the presilphiperfolane skeleton). A detailed study of the *Bcbot4Δ* null mutant has been undertaken in order to study the metabolic fate of the presilphiperfolan-8-ol intermediate biosynthesized by this organism and in particular by this strain. As a result three new presilphiperfolanes and three new cameroonanes have been identified. The results suggest that the absence of the oxygen function at C-11 of the presilphiperfolane skeleton permits rearrangement to a cameroonane whilst hydroxylation at C-11 precludes this rearrangement. It is possible that the interactions of the C-11 hydroxylated derivatives perturb the stereo-electronic requirements for the migration of the C-11:C-7 σ bond to C-8.

**Introduction**

Fungi from the genus *Botrytis* are widespread, having been identified in cold regions such as Alaska and Canada as well as in sub-tropical regions such as Egypt. They are necrotrophic parasites which cause serious economic losses to commercial crops. The polyphagous species *Botrytis cinerea* has been identified as a pathogen of more than 200 plant species, often attacking damaged parts of the plant before spreading to healthy tissues. The most common symptoms involve the development of necrotic lesions on the leaves. The initiation and progress of the infection by *B. cinerea* involves a complex sequence of biological events including both enzymatic attack and the production of phytotoxic secondary metabolites. However in accordance with the broad range of host of this pathogen, there is no evidence for the production of host-specific toxins. The toxins produced by *B. cinerea* not only reproduce the symptoms of a fungal infection but have also been detected in plant tissues infected by *B. cinerea*. Two major families of phytotoxic metabolites are sesquiterpenes related to botrydial (1) and the polyketides, 3-acetylbotcinic and 3-acetylbotcinic acids (2, 3) and their botcinin (4, 5) relatives, Scheme 1. Botrydial (1) is produced during the infection of a plant and induces chlorosis and cell collapse, 3-Acetylbotcinic acid (2) and its derivatives also induce chlorosis and necrosis and have some anti-fungal activity, (Scheme 1).

The sequencing of the *B. cinerea* genome has led to the identification of the botrydial biosynthetic gene cluster, Fig. 1. Such gene clusters are typical of the secondary metabolism in filamentous fungi. This cluster consists of seven genes (*BcBOT1* to *BcBOT7*) coding for a sesquiterpene cyclase (*BcBOT2*), an acetyltransferase (*BcBOT5*), a transcription...
The mutant \( \text{Bcbot4}\Delta \) was grown on a malt agar medium (1 L) for 10, 17 and 21 days. A detailed TLC analysis of the extracts revealed significant differences between the 10 and 17 day fermentations when compared to a 5 day fermentation. The mutant was then grown on a malt agar medium (6 L) for 17 days at 23 °C under white light. The metabolites were recovered from the medium by extraction with ethyl acetate and the constituents of the extract (6.7 g) were separated by chromatography. A small amount of compound 8 was obtained from the less polar fraction. The polyketides 2–7 and 9–15 were also isolated and identified by comparison of their spectroscopic data with those of authentic samples. Six new compounds (16–21) were isolated from the fractions of medium polarity and purified by further chromatography including HPLC. The \(^1\)H and \(^{13}\)C NMR spectra suggested that they possessed sesquiterpenoid structures.

The HRMS and \(^{13}\)C NMR data for compound 16 which were obtained as a colourless crystalline solid, corresponded to a molecular formula of \(C_{15}H_{26}O_3\). It possessed IR absorption at 3430 cm\(^{-1}\) (OH). The \(^{13}\)C NMR spectrum, aided by DEPT and HSQC experiments (see ESI Table S1†) exhibited three methyl group signals, five methylenes including one hydroxymethyl group, four methines including one secondary alcohol and three quaternary carbons including a tertiary alcohol. The three methyl group signals in the \(^1\)H NMR spectrum were a doublet and two singlets, whilst the hydroxymethyl group signal only contained a geminal coupling. The secondary alcohol resonance was a doublet of double-doublets. These data suggested that compound 16 was a dihydroxypresilphiperfolan-8-ol. The HMBC spectrum revealed correlations between the signal for the secondary alcohol at \(\delta \text{H} 3.25\) and \(\delta \text{C} 7\) and \(\delta \text{C} 9\) and \(\delta \text{C} 11\) and \(\delta \text{C} 15\) and between the hydroxymethyl group at \(\delta \text{H} 2.34\) indicating a \(\beta\) configuration for this hydroxyl group. Finally the structure was confirmed by X-ray crystallography (Fig. 2). On acetylation with acetic anhydride in pyridine, compound 16 formed a diacetate \(16a\) (OAc \(\delta \text{H} 2.05\) and 2.08) in which the signals for H-14 and H-10 were shifted to \(\delta \text{H} 4.0\) and 4.6 respectively.

The minor metabolites 17 and 18 showed \(^1\)H NMR signals which were similar to those of compound 16 retaining the signals for the three methyl groups, the tertiary alcohol and the hydroxymethyl group. The principle difference in the spectra of 17 was the lack of the CH(OH) signal at \(\delta \text{H} 3.25\) and the presence in the \(^{13}\)C NMR spectrum of a carbonyl signal at \(\delta \text{C} 213.7\). The H-11 signals were deshielded to \(\delta \text{H} 2.34\) and 2.52. Consequently compound 17 was the oxidation product of 16 at C-10, a conclusion supported by 2D COSY, HMBC and HSQC experiments (see Table S2, ESI†). The major difference in the NMR spectra of compound 18 compared to that of com-
compound 16 was the presence of alkene signals at δH 4.94 (dd, J 2.1 and 2.5 Hz) and δC of 148.7 and 116.2 corresponding to a trisubstituted alkene. The location of the alkene between C-1 and C-2 was established by the deshielding of the methyl group doublet and the H-3 signals at δH 2.40 (1H, dd, J 17 and 2.1 Hz) and 2.25 (1H, dt, J 17 and 2.5 Hz). In the HMBC spectrum (see Table S3, ESIF) there were correlations between H-2 and C-3, C-4 and C-8 and between H-15 and C-1. Thus the structures of 16–18 were presilphiperfolan-8α,10β,14-triol (16), 8α,14-dihydroxy-presilphiperfolan-10-one (17) and 8α,10β,14-trihydroxy-presilphiperfolan-1-ene (18).

The HRMS and 13C NMR data for compound 19 corresponded to a molecular formula, C15H26O3. The 1H and 13C NMR spectra, (Table S4, ESIF) revealed the presence of one secondary methyl group and two tertiary methyl groups, four methylene and one hydroxymethyl group, two secondary alcohols and two further methine carbons and three quaternary carbons. Whilst there were similarities to the spectra of the presilphiperfolanoltriol 16, there was a major difference in the absence of the tertiary alcohol. However the spectra were in accordance with a cameroonane carbon skeleton. Cameroonan-7α-ol has previously been obtained by the rearrangement of presilphiperfolan-8α-ol.21,22 The 1H NMR signal of one of the secondary alcohols was a singlet (δH 3.89) indicating that this alcohol was situated between two quaternary carbons typical of the cameroonan-7α-ol. The other secondary alcohol was a doublet of double-doublets (δH 3.72, ddd, J 11, 9.7 and 6.0 Hz). Two of these couplings were assigned to a methylene (δH 1.73 and 1.65). Apart from their geminal coupling (J 12.2 Hz), these resonances possessed no additional couplings. Together with the HMBC spectra, this was consistent with the location of this hydroxyl group at C-10 (see Table S4, ESIF). The stereochemistry of the alcohols was established by a NOESY correlation between the H-10 resonance and H-15, between H-7 and H-12 and H-9, and between H-14 and H-1. Furthermore when compared to the 13C NMR spectrum of cameroonan-7α-ol there were γ-gauche shielings by the C-10 β hydroxyl group of the resonances of C-1, C-8 and C-15 and by the C-14 hydroxyl group of C-5. This compound formed a triacetate (19a), the spectra of which were consistent with the formulation of the parent alcohol as cameroonan-7α,10β,14-triol (19).

The 13C NMR spectrum (Table S5†) of compound 20 contained signals assigned to four methyl groups, three methylenes, five methines, three of which were secondary alcohols and three quaternary carbon atoms. The pattern of the 1H NMR signals was similar to that of compound 19 particularly in the presence of the singlet assigned to H-7. However the signal assigned to H-10 was replaced by a double-doublet (δ 3.49, J 8.6 and 9.1 Hz) and a doublet (δ 3.81, J 9.1 Hz) which were assigned to two secondary alcohols. Their vicinal relationship was revealed by the COSY spectrum whilst their location at C-2 and C-3 followed from HMBC correlations between H-2 and C-9 and C-3 and between H-3 and C-12 and C-5. Their stereochemistry followed from the magnitude of the vicinal coupling constants H-1:H-2 (J 8.6 Hz) and H-2:H-3 (J 9.1 Hz) which corresponded to trans relationships between these protons. There were noe correlations between H-2 and H-9 and H-12 and between H-3 and H-1, H-5α and H-14. The stereochemistry of the alcohol at C-7 followed from the noe enhancements of H-11β and H-13 on irradiation of H-7. Acetylation of the triol gave a triacetate (20a) with NMR data (see Experimental) that was consistent with the structure of cameroonan-2α,3β,7α-triol for the parent alcohol. It is interesting to note that the stereochemistry of this vicinal diol (20) corresponds to that formed from presilphiperfolan-8α-ol (20c) prior to the cleavage of the five-membered ring to form botrydial (1). The oxygenases that mediate this step are coded by the genes BcBOT1 and BcBOT3. Molecular models reveal a structural similarity between the presilphiperfolan-8α-ol substrate and a cameroonan-7α-ol in which a presilphiperfolanol 11α-hydroxyl group and a cameroonan-7α-hydroxyl group would also be juxtaposed.

Compound 21 was the most polar metabolite. The 1H and 13C NMR spectra, (Table S6†) contained signals assigned to two tertiary methyl groups, five methylenes, three methines and three quaternary carbon atoms and two carbonyl groups at δC 180.6 and 175.7. These were assigned to a carboxylic acid and an ester function. The characteristic cameroonan-7α-ol, H-7, singlet was shifted to δH 4.27 and the presence of IR absorption at 1810 cm⁻¹ was indicative of the presence of a β-lactone. There were HMBC correlations between H-12 and C-3, C-4, C-5 and C-8 and between H-13 and C-5, C-6, C-7 and C-14 thus locating the carboxyl group at C-15. Compound 21 was therefore assigned the structure of 7α-hydroxycameroonan-14,15-dioic acid 14,7-lactone.

Biologically these cameroonane metabolites may be formed by the rearrangement of presilphiperfolan-8α-ol which has laboratory precedent.22 The loss of the 8α-hydroxyl group may generate a C-8 carbocation (A) which may be followed by the rearrangement of the C-11:C-7 bond to C-8 to yield a C-7 carbocation (B), Scheme 3. This is then discharged by a hydroxyl group to form the cameroonan-7α-ol (22). The geometrical requirements for this rearrangement have been examined. An X-ray crystal structure of the p-nitrobenzoate of presilphiperfolan-8α-ol revealed that there is a trans-relationship
between the departing \(\alpha\)-hydroxyl group and the migrating C-11:C-7 bond in a chair six-membered ring. It is likely that this rearrangement leads to the formation of the cameroonanes in *B. cinerea*.

In the same line compound 19, Scheme 3, should be formed from the isolated compound 16, while compound 20 must arise from the rearrangement of the hypothetical precursor 20b.

Compounds with the cameroonane carbon skeleton have not been detected previously as the metabolites of *B. cinerea*. Fermentation of the intact *B. cinerea* for 15 and 17 days led to the isolation of botryanes and the presilphiperfolane 20c, Scheme 2, the precursor of botrydial (1) by oxidative cleavage of the C10–C15 bond. The presence of the C-11 hydroxyl group (C-4 in botryane numbering, see 8) which is introduced by the enzyme system mediated by *BcBOT4*, appears to inhibit the rearrangement. It could be that transannular hydrogen bonding to the C-8 hydroxyl group and interactions between the C-11 hydroxyl group and the methyl groups at C-4, lead to a distortion of the chair form of the six-membered ring and so reduces the facility with which the ring-contraction occurs.

**Experimental**

**General experimental procedures**

Purification by semipreparative and analytical HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a differential refractometer detector (RI-7490). A LiChrospher® Si 60 (5 µm) LiChroCart® (250 mm × 4 mm) column and a LiChrospher® Si 60 (10 µm) LiChroCart® (250 mm × 10 mm) were used in isolation experiments. Silica gel (Merck) was used for column chromatography. TLC was performed on Merck Kieselgel 60 F254, 0.25 mm thick. Melting points were measured with a Reichert-Jung Kofler block and are uncorrected. Optical rotations were determined with a digital polarimeter. Infrared spectra were recorded on a FT-IR spectrophotometer and reported as wave number (cm\(^{-1}\)). \(^1\)H and \(^13\)C NMR measurements were recorded on Agilent 500 and 600 MHz spectrometers with SiMe\(_4\) as the internal reference. Chemical shifts were referenced to CDCl\(_3\) (\(\delta_H 7.25, \delta_C 77.0\)). NMR assignments were made using a combination of 1D and 2D techniques. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quarter; quint = quintuplet; sext = sextuplet; m = multiplet, br = broad. High-Resolution Mass Spectroscopy (HRMS) was performed either with a double-focusing magnetic sector mass spectrometer in a QTOF mass spectrometer in positive or negative ion ESI or APCI modes (APGC+ for samples analysed by GC chromatography).

**Fungal material**

**Fungal strains.** Strain B05.10 of *Botrytis cinerea* Pers. Fr. was isolated from a *Vitis* field sample.

**Media and culture conditions.** Standard procedures\(^{19}\) for culturing and maintaining of *B. cinerea* strains and the mutant were performed at 23 °C with 12 h daylight per day. Cultures
were grown on malt agar medium (20 g per L malt extract, 5 g per L yeast extract, 15 g per L agar), and minimal medium (20 g per L glucose, 2 g per L NaNO₃, 1 g per L K₂HPO₄, 50 mg per L MgSO₄·7H₂O, 500 mg per L KCl, 10 mg per L FeSO₄·7H₂O, 15 g per L agar). The transformation medium was a minimal medium supplemented with saccharose (20 g per L glucose, 200 g per L saccharose, 2 g per L NaNO₃, 1 g per L K₂HPO₄, 500 mg per L MgSO₄·7H₂O, 500 mg per L KCl, 10 mg per L FeSO₄·7H₂O, 15 g per L agar). The bcbot4 mutant was selected with nourseothricin fungicide (Werner Bioagent, Germany- 70 µg ml⁻¹).

For the isolation of metabolites, the null mutant Δbcbot4 of Botrytis cinerea was cultured in 200 mm Petri dishes containing 100 mL of malt agar medium (6 L) for 17 days at 23 °C under white light. After the growth time, the media were extracted with ethyl acetate. The extracts were dried over sodium sulfate, and the solvent was evaporated to give 6.7 g of extract.

**Gene deletion of the cytochrome P450-encoding gene bcbot4**

The B. cinerea gene bcbot4 (GenBank: AV277723.2) was deleted by homologous recombination. The 5' genomic non-coding regions of bcbot4 (1442 pb) was amplified by PCR. In the same way, we obtained the 3' regions of bcbot4 (866 pb) and the nourseothricin resistance gene (nat). For each gene, a Knock-Out (KO) cassette consisting of the 5' region of bcbot4, the nat resistance gene and the 3' region bcbot4 was generated by double-joint PCR fusion as previously described for B. cinerea. All the PCR primers have been reported. Additional run at 25 °C in 200 mm Petri dishes containing 100 mL of malt agar medium (6 L) for 17 days at 23 °C under white light. After the growth time, the media were extracted with ethyl acetate. The extracts were dried over sodium sulfate, and the solvent was evaporated to give 6.7 g of extract.

**Isolation of the metabolites**

Fractionation of the crude extract was carried out on a silica column to give six fractions using 100% hexane (5.3 mg), hexane : ethyl acetate (70 : 30) (1.7 g), hexane : ethyl acetate (40 : 60) (2.1 g), hexane : ethyl acetate (10 : 90) (1.25 g), ethyl acetate : methanol (70 : 30) (1.3 g) and 100% methanol (303.8 mg).

In addition to the known compounds 2–15, which were obtained from the fractions of medium polarity, the sesquiterpenes presilphiperfolan-8α,10β,14-triol (16) (93 mg), 8α,14-dihydroxypresilphiperfolan-10-one (17) (1.5 mg), 8α,10β,14-trihydroxypresilphiperfol-1-ene (18) (1 mg), cameroon-7α,10β,14-triol (19) (102 mg) and 7α-hydroxycameroonane-14,15-dioic acid 14-7-lactone (21) (6.71 mg) were isolated and characterized. Compounds 16 (2.03 mg), 19 (1.27 mg) and 20 (1.54 mg) were acetylated. For acetylation, compounds were dissolved in the minimum quantity of dry pyridine (0.5 mL), and acetic anhydride (30 mg) was added to the solution. The mixture was kept at room temperature with stirring for 24 h. The solvent was evaporated to give derivatives 16a (2.20 mg), 19a (1.32 mg) and 20a (1.49 mg).

**Presilphiperfolan-8α,10β,14-triol (16).** Colourless solid; mp 162 °C; [α]D = -1.02 (c 10.5 in CH₃OH); IR (film) νmax 3293, 2948, 2874, 1455, 1047 cm⁻¹; 1H NMR (500 MHz, CD₃OD) δ 3.35 (2H, d superimposed, J = 10 Hz, H-14), 3.23 (1H, ddd, J = 11, 9.4, 3.4 Hz, H-10), 2.34 (1H, m, H-2), 2.02 (1H, d, J = 12.1 Hz, H-5), 1.92 (2H, m, H-2', H-3), 1.86 (1H, m, H-7), 1.82 (1H, m, H-11), 1.59 (1H, ddd, J = 12, 11 Hz, H-11'), 1.43 (1H, m, H-1), 1.41 (1H, m, H-9), 1.34 (3H, s, H-12), 1.16 (3H, s, H-13), 1.13 (1H, m, H-3'), 1.01 (1H, d, J = 12.1 Hz, H-5'), 0.97 (3H, d, J = 5.9 Hz, H-15); 13C NMR (125 MHz, CDCl₃) δ 95.3 (C, C-8), 76.3 (CH, H-10), 72.5 (CH₂, H-14), 57.9 (C, C-4), 54.4 (C, C-6), 47.6 (CH, C-9), 45.9 (CH, C-7), 44.9 (CH, C-1), 43.5 (CH₂, C-5), 36.9 (CH₂, C-11), 34.3 (CH₂, C-11), 34.1 (CH₃, C-3), 29.0 (CH₃, C-12), 22.8 (CH₃, C-13), 18.0 (CH₃, C-15); HRMS (ESI): calcd for C₁₃H₂₆O₃ [M – 2H₂O + H⁺]⁺ 219.1749, found 219.1744.

**10β,14-Diacetoxypresilphiperfolan-8α-ol (16a).** Colourless oil, 1H NMR (500 MHz, CDCl₃) δ 4.61 (1H, td, J = 11.1, 3.5 Hz, H-10), 4.0 (s, 2H, H-14), 2.41 (1H, m, H-2'), 2.08 (s, 3H, CH₃CO), 2.05 (s, 3H, CH₃CO), 1.98 (1H, m, H-2'), 1.94 (1H, d, J = 12.1 Hz, H-5a), 1.90 (1H, m, H-3), 1.88 (1H, m, H-9), 1.88 (1H, m, superimposed, H-11), 1.68 (1H, td, J = 11.0, 6.2 Hz, H-1), 1.58 (1H, m, H-11), 1.46 (1H, ddd, J = 11.2, 9.1, 3.9 Hz, H-7), 1.34 (3H, m, H-12), 1.24 (1H, m, H-3'), 1.21 (3H, m, H-13), 1.14 (1H, d, J = 12.1 Hz, H-5b), 0.89 (3H, d, J = 6.3, 3H, H-15). 13C NMR (125 MHz, CDCl₃) δ 170.9 (C, CH₃CO), 170.7 (C, CH₃CO), 94.44, (C, C-8), 77.11 (CH, C-10), 74.7 (CH₂, C-14), 56.13 (C, C-4), 51.64 (C, C-6), 46.9 (CH, C-7), 44.6 (CH, C-9), 43.61 (CH₂, C-5), 41.0 (CH-1), 33.23 (CH₂, C-2), 33.07 (CH₂, C-3), 32.4 (CH₂, C-11), 28.32 (CH₃, C-12), 22.53 (CH₃, C-13), 21.2 (CH₃CO), 21.0 (CH₃CO), 17.20 (CH₃, C-15); HRMS (APGC): calcd for C₁₃H₂₆O₃ [M + H⁺]⁺ 339.2171, found 339.2162.

**X-ray crystallographic analysis of 16.** Crystals of 16 were obtained from n-hexane/EtOAc by slow evaporation. X-ray diffraction data were recorded on a Bruker Smart CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 298(1) K. Data collection was based on three ω-scan runs (starting = -34°) at values of ϕ = 0°, 120°, and 240° with the detector at 2θ = -32°. For each of the runs, 606 frames were collected at 0.3° intervals and 10 s per frame. An additional run at ϕ = 0° of 100 frames was collected to improve redundancy.

The diffraction frames were integrated using the program SAINT, and the integrated intensities were corrected for Lorentz polarization effects and absorption with SADABS. The structure was solved by direct methods and refined to all 3237 unique Fₐ values by full matrix least-squares calculations using SHELXL-97. All the hydrogen atoms, except the hydrogens of the –OH groups, were placed at idealized positions and refined as rigid atoms. The positions of the OH hydrogens are found in the diffraction map and refined as rigid atoms. Final R₁ indices [2699 with I > 2σ(I)] 0.0465, wR₂ = 0.1074. R indices (all data) R₁ = 0.0578, wR₂ = 0.1150. Space group: P2₁2₁2₁, Z = 4, F(000) = 560, µ = 0.081 mm⁻¹.
Crystallographic data of presilphiperfolan-8α,10β,14-triol have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1547406.

8α,14-Dihydroxypresilphiperfolan-10-one (17). Colourless oil; [α]D20 = -33.75 (c 0.20 in CHCl3); IR (film) νmax 3303, 2918, 2874, 1707, 1455, 1037 cm⁻¹; 1H NMR (600 MHz, CDCl3) δ 3.44 (2H, s(br), H-14, H-14′), 2.52 (1H, dd (br), J = 13.1 Hz, H-11β), 2.50 (1H, m, H-2′), 2.43 (1H, dq, J = 12.4, 6.3 Hz, H-9), 2.34 (1H, dd, J = 13.1, 8.7 Hz, H-11α), 2.20 (1H, m, H-7), 2.17 (1H, d, J = 12.1 Hz, H-5α), 2.05 (1H, m, H-2), 1.71 (1H, m, H-1), 1.98 (1H, m, H-3), 1.54 (3H, s, H-12), 1.26 (1H, m, H-3′), 1.14 (1H, d, J = 12.1 Hz, H-5β), 1.14 (3H, s, H-13), 0.98 (3H, d, J = 6.4 Hz, H-14). 13C NMR (150 MHz, CDCl3) δ 213.7 (C, C-10), 92.4 (C, C-8), 71.5 (CH2, C-14), 57.8 (C, C-4), 54.0 (C, C-6), 48.13 (CH, C-9), 48.09 (CH, C-1), 46.2 (CH2, C-7), 42.2 (CH2, C-11), 41.7 (CH3-C, 3), 34.9 (CH2, C-2), 32.7 (CH, C-3), 28.1 (CH2, C-12), 22.5 (CH2, C-13), 13.5 (CH3-C-15). HRMS (ESI): C15H23O3 [M - H]⁻ 251.1647, found 251.1655.

Cameronano-2α,3β,6α-tri-20 (Amorphous solid, [α]D20 = -26.8 (c 1.32 in CHCl3); IR (film) νmax 3381, 2949, 2858, 1456, 1378, 1040, 756 cm⁻¹; 1H NMR (500 MHz, CDCl3) δ 3.81 (1H, dbr, J = 9.1 Hz, H-3), 3.49 (1H, dd, J = 8.6, 9.1 Hz, H-2), 3.33 (1H, s, H-7), 2.10 (1H, dd, J = 8.6, 3.7 Hz, H-1), 1.94 (1H, m, H-11α), 1.90 (1H, m, H-11α), 1.83 (1H, d, J = 13.5 Hz, H-5α), 1.73 (1H, m, H-10β), 1.55 (1H, m, H-11β), 1.34 (1H, m, H-10α), 1.29 (1H, d, J = 13.5 Hz, H-5β), 1.03 (3H, s, H-13), 1.03 (3H, s, H-14), 1.01 (1H, d, J = 6.9 Hz, H-15), 0.95 (3H, s, H-12). 13C NMR (125 MHz, CDCl3) δ 90.4 (CH, C-7), 86.5 (CH, C-3), 82.9 (CH, C-2), 64.10 (C, C-8), 54.8 (CH-1), 52.85 (CH2, C-5), 49.8 (C, C-4), 43.7 (C, C-6), 38.61 (CH, C-9), 35.7 (CH2, C-11), 34.9 (CH2, C-10), 29.4 (CH3-C, 13), 24.3 (CH3-C, 14), 20.3 (CH3-C, 15), 20.0 (CH2, C-13). HRMS (EI): calcd for C21H21O8 [M + H⁺]: 355.1060, found 355.1064.

Cameronano-7α,10β,14-tri-20 (Amorphous solid, 1H NMR (500 MHz, CDCl3) δ 5.18 (1H, d, J = 6.7 Hz, H-3), 4.97 (1H, dd, J = 6.7 Hz, H-2, 4.87 (1H, s, H-7), 2.12 (1H, d, J = 13.9 Hz, H-5α), 2.14 (3H, s, CH3-CO), 2.08 (1H, m, H-1), 2.05 (3H, s, CH3-CO), 2.05 (3H, s, CH3-CO), 1.99 (1H, m, superimposed, H-9), 1.95 (1H, m, superimposed, H-10α), 1.80 (1H, ddd, J = 12.7, 6.5, 4.3 Hz, H-10β), 1.64 (1H, m, H-11), 1.39 (1H, d, J = 13.9 Hz, H-5β), 1.10 (3H, s, H-14), 1.22 (1H, m, H-11), 1.02 (3H, s, H-12), 1.0 (3H, s, H-13), 0.96 (3H, d, J = 6.8 Hz, H-15). 13C NMR (125 MHz, CDCl3) δ 170.4(CH2CO), 170.4(CH2CO), 170.3(CH2CO), 89.9 (CH, C-7), 85.3 (CH, C-3), 83.2 (CH, C-2), 65.7 (C, C-8), 55.9 (CH, C-1), 52.4 (CH2, C-5), 50.6 (C, C-4), 42.1 (C, C-6), 40.3 (CH, C-9), 35.6 (CH2, C-10), 33.1, 30.3 (CH3-C, 14), 25.0 (CH2, C-12), 21.1 (CH2, CO2), 21.2 (CH2, CO2), 20.9 (CH2, C-15), 19.8 (CH3, C-13). HRMS (APGC): calcd for C25H24O18 [M + H⁺]: 381.2277, found 381.2264.

7α-Hydroxycameronano-14,15-dioic acid 14-7-lactone (21). Colourless solid; mp 114 °C; [α]D20 = -28.97 (c 0.58 in CHCl3); IR (film) νmax 3423, 2959, 1811, 1698, 1456, 1239, 913, 846 cm⁻¹; 1H NMR (500 MHz, CDCl3) δ 4.27 (1H, s, H-7), 3.01 (1H, dt, J = 6.7, 8.3 Hz, H-1), 2.52 (1H, dt, J = 6.7, 8.3 Hz, H-9), 2.28 (1H, d, J = 14.4 Hz, H-5), 2.14 (2H, overlap, H₂-2′, H-10), 2.04 (1H, m, H-10′), 1.90 (1H, m, H-3), 1.80 (1H, m, H-11′), 1.64 (1H, dd, J = 12.7, 6.7 Hz, H-3′), 1.52 (1H, m, H-2′), 1.44 (1H, dd, J = 13.2, 6.4 Hz, H-11α), 1.40 (3H, s, H-13), 1.37 (1H, d, J = 14.4 Hz, H-5′), 1.07 (3H, s, H-12). 13C NMR (125 MHz, CDCl3) δ 180 (C, C-15), 175.7 (C, C-14), 93.6 (CH, C-7), 65.3 (C, C-8), 62.3 (C, C-6), 54.8 (C, C-4), 51.7 (CH, C-9), 50.7 (CH, C-1), 45.1 (CH2, C-5), 42.6 (CH2, C-3), 33.0 (CH2, C-10), 32.5 (CH3-C-2), 31.1 (CH3-C-11), 25.1 (CH3-C-12), 18.0 (CH3, C-13). HRMS (ESI): calcd for C21H21O9 [M - CO2 + H⁺]: 321.1542, found 321.1541.
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Notes and references