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The role of botrydienediol in the biodegradation of the sesquiterpenoid phytotoxin botrydial by *Botrytis cinerea*

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Abstract—The biotransformation of botrydienediol (**6**) labelled with deuterium on carbons C-10 and C-15 has been studied. This has led to modification of some previous assumptions about the biodegradative route of botrydial. The [10-^{2}H,15-^{2}H]-botry-1(9)-4-diendiol (**12**) was transformed into dehydrobotrydienediol derivatives **13**–**15** but it was not incorporated into secobotryane skeleton (**7**). In addition, three new sesquiterpenoids have been isolated, which shed further light on the secondary metabolites of *Botrytis cinerea*. From the point of view of persistence of these toxins in the food chain, the easy biotransformation and different biodegradative routes of botrydial (**1**), seem to indicate that the toxin may not persist in the plant for a long time as it will be metabolized by the fungi and the plant.

1. Introduction

The ascomycete *Botrytis cinerea* Pers.:Fr. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel] is a phytopathogenic fungus that grows as a grey mould on a variety of commercial crops causing serious economic losses.[1] A number of phytotoxins have been isolated from this fungus.[2] The best known and most active of these metabolites is botrydial (**1**), which possesses the sesquiterpenoid botryane skeleton,[3] it is responsible for the typical lesions of the fungal infection and it plays an important role in the pathogenicity of the organism in vivo.[4,5]

Botrydial (**1**) has recently been detected in ripe fruits of *Capsicum annuum* and in the leaves of *Phaseolus vulgaris* and *Arabidopsis thaliana* that have been wounded and inoculated with a conidial suspension of *B. cinerea*.[6]

The regulatory effect of botrydial (**1**) on the growth of *B. cinerea* and its biodegradation by the fungus has been reported.[7] Fungal growth ceases when the concentration of botrydial reaches a particular level. The fungus transformed botrydial (**1**) to the less active phytotoxins dihydrobotrydial (**3**) and secobotrytrienediol (**7**). It is only after its detoxification to the less harmful compounds **3**–**7** that fungal growth resumes.[7] This may have implications for our understanding of the progress of the fungal infection of a plant and, additionally, from the point of view of its persistence in the food chain, to understand the fate of this toxin in fruits and vegetables infected by this phytopathogenic fungus. Consequently, the study on biodegradation pathway of the major toxin, botrydial, it is of interest. In the light of our results two main biodegradation and detoxification pathways for botrydial (**1**) were proposed and are set out in Scheme 1 (routes a and b).[7] In this paper we report the results of investigations on the biotransformation of the putative biosynthetic intermediate botrydiendiol (**6**) by *B. cinerea* and some considerations of the secondary metabolism of the fungus.

2. Results and discussion

As indicated above, the biodegradative route leading to secobotrytrienediol (**7**) has come to our attention because this transformation could have implications for the progress of the fungal infection of a plant and for the safety of the food chain.

In addition, the biosynthesis of metabolite secobotrytrienediol (**7**) is regulated by pH.[7] The formation of **7** may involve an electrocyclic ring opening reaction from the diene **5** or its reduced derivative **6**. This is an interesting process in

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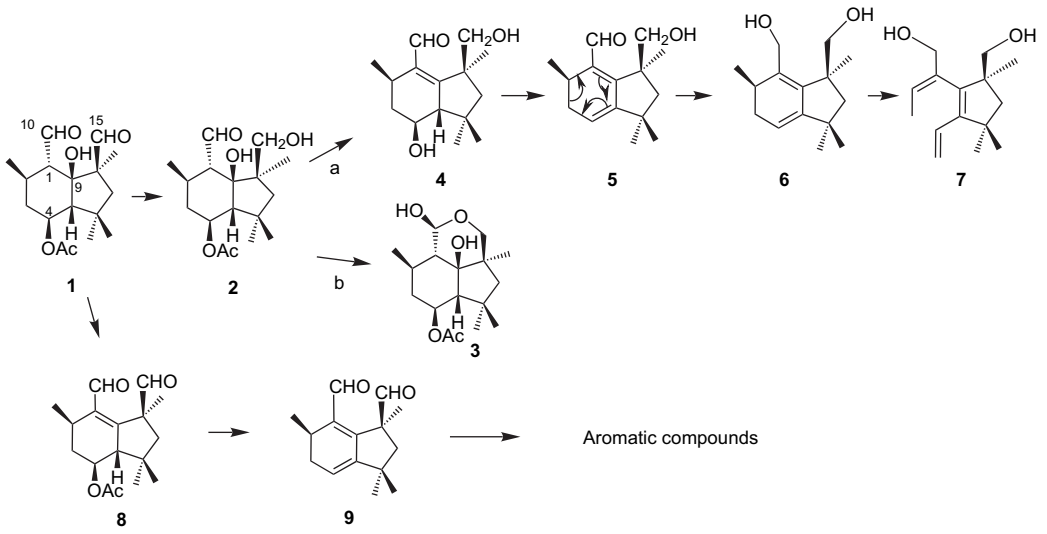


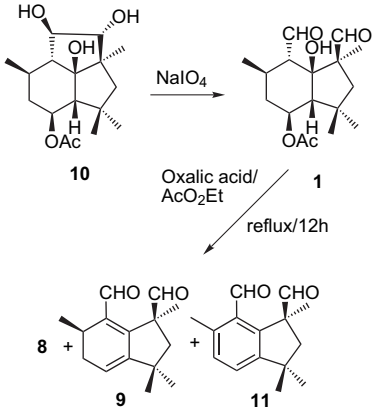
Scheme 1.

biological systems and has a parallel in the formation of precalciferol.[8]

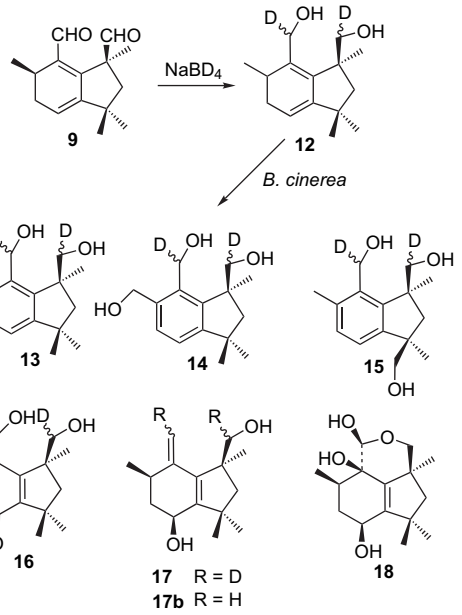
In order to gain a greater understanding of the secondary metabolism within the fungus *B. cinerea*, the biotransformation of the botrydienediol (**6**) labelled with deuterium on carbons C-10 and C-15 has been studied.

Compound (**9**) was obtained from the natural probotryane (**10**) by the bio-mimetic chemical transformation indicated in Scheme 2. Compound **10**, obtained from fermentation of *B. cinerea*, was treated with periodate to yield botrydial (**1**) (60%). Reaction of **1** with oxalic acid gave compounds **8**,[2,5] **9**[2,9] and **11**[2,9] with 15, 64 and 10% yield, respectively (Scheme 2). The reduction of **9** with deuteriated sodium borohydride yielded the deuteriated botrydiendiol (**12**) (Scheme 3).

The deuteriated compound **12** was fed to two fermentations one of which was buffered at pH 7 whilst the other was allowed to grow at the natural pH. After three days, from inoculation, (see Section 3), the mycelium of each experiment was filtered. The broths were saturated with sodium chloride and extracted four times with ethyl acetate. The solvent was evaporated and the residue was purified by chromatography



Scheme 2.



Scheme 3.

on a Si gel column. Final purification was carried out by means of semi-preparative HPLC.

The experiment using a buffered medium afforded, in addition to the deuteriated compounds **13–15**, two new deuteriated compounds 1-hydroxy-4-oxo-[10-^{2}H, 15-^{2}H]-botry-5(9)-endiol (**16**) and botry-[1(10),5(9)-^{2}*H*]-dien-[15-^{2}*H*]-ol (**17**) and an undeuteriated new metabolite 1β,4β-dihydroxy-5(9)-ene dihydrobotrydial (**18**). Compounds **13–17** were also obtained from the fermentation grown at the natural pH (Scheme 3). The structures of the metabolites were established on the basis of their one-dimensional and two-dimensional NMR spectra (^{1}H, ^{2}H, ^{13}C, HSQC and HMBC).

Compounds **13–15** showed an average deuterium incorporation of 79% and a characteristic pattern of signals, in their ^{1}H and ^{13}C NMR, corresponding to the aromatic botryane derivatives isolated previously from *B. cinerea* and previously

reported.[10] Although they could be considered as artefacts from the aromatization of diene **12** in an acidic medium, the isolation of these compounds from the biotransformation in buffered medium would indicate that compounds **13–15** arise from a biotransformation of **12** by the fungus.

Interestingly, two new compounds with a previously unknown functionality on carbon C-1 were isolated. Compound **16** was isolated as a diastereomeric mixture of deuteriated derivatives, displaying a 44% deuterium incorporation. The HREIMS of **16** showed a molecular ion corresponding to the molecular formula $C_{15}D_2H_{22}O_4$. The IR spectrum possessed absorption bands at 3375, 2930, 1660, 1355, 1059 cm^{-1}, indicating that an unsaturated ketone and hydroxyl groups were present. The ^{13}C NMR spectrum showed 15 signals arising from four methyls, four methylenes, one methines and six quaternary carbon atoms, including a ketonic carbonyl group at δ 197 and a double bond at δ 145.0 and 169.1.

The ^1H NMR spectrum of the product showed a typical pattern of botryane signals from which the stereochemistry has already been established.[2] The principal differences were the absence of the signal corresponding to a hydroxyl group at C-4 and, in this deuteriated compound, the signals (singlets) corresponding to H-10 and H-15, at δ 3.72–3.76 and 3.53, respectively. These signals could be superimposed with those signals corresponding to the compound without deuterium. The location of a carbonyl group at C-4 was confirmed by the HMBC correlation of the corresponding carbonyl signal with the methine proton H-2, at δ 2.39. Furthermore, the HMBC experiment also provided information on the location of the hydroxyl group. The long-range correlations of the carbon at δ 75.6 with the methyl doublet at C-2 clearly marked the presence of hydroxyl group at C-1.

The stereochemistry of hydroxyl group was inferred from the observed downfield shift of the methyl group on C-2, which was consistent with a β disposition for a hydroxyl group on C-1. The assignment of the signals was supported by ^1H–^1H COSY, HSQC and HMBC experiments and ^2H NMR spectrum, and was consistent with the proposed structure **16**.

Compound **17** displayed 79% deuterium incorporation and it was isolated as a diastereomeric deuteriated mixture. Its molecular formula $C_{15}D_2H_{22}O_2$ was established on the basis of HREIMS data from the *m/z* 238.1895. The ^{13}C NMR spectrum showed 15 signals arising from four methyls and four methylenes, including a methylene on a double bond; two methines, one corresponding to an oxygenated carbon; and five quaternary carbon atoms. Three quaternary carbons and one methine were assigned to two double bonds.

The ^1H NMR spectrum of the product showed a typical pattern of botryane signals. The principal difference was the presence of signals at δ 4.95 and 5.13 corresponding to an exocyclic double bond, which was easily located at C-1 by an HMBC experiment. The signal at δ 4.39 (1H, dd, H-4) was assigned to the geminal proton to a hydroxyl group on C-4. The HSQC and HMBC experiments clearly established the presence of the fully substituted C-5–C-9 double bond and exocyclic double bond between C-1–C-10. Signals at δ 2.6 and 4.39 were assigned to H-2 and H-4, respectively. The ^2H NMR spectrum was consistent with the proposed structure **17**.

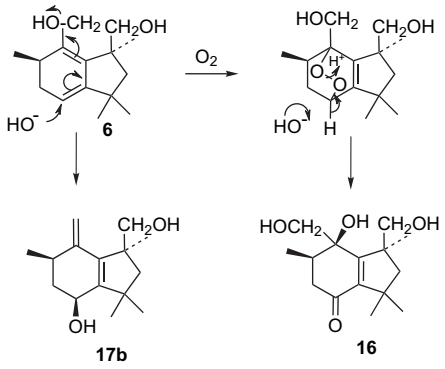
This compound had previously been isolated in very small amount from an experiment on the biotransformation of diisophorone by *B. cinerea*.[11] Its structure was not established at that time. The spectroscopic data of this compound **17b** are now included in Section 3.

The undeuteriated compound **18** was obtained from the experiment with buffered medium. The HREIMS showed a molecular ion corresponding to molecular formula $C_{15}H_{24}O_4$. The IR spectrum possessed absorption bands at 3410, 2954, 1639, 1035 and 751 cm^{-1} indicating that hydroxyl groups and an alkene were present. The ^{13}C NMR spectrum showed signals arising from four methyls, three methylenes, three methines and five quaternary carbon atoms, including a fully substituted double bond. The ^1H NMR spectrum shows a pattern of the signals comparable to those of dihydrobotrydial (**3**). However, the signal corresponding to H-10 appeared as a singlet and the signal of H-4 was shifted upfield suggesting the introduction of a new hydroxyl group in C-1 and a double bond on C-5–C-9.

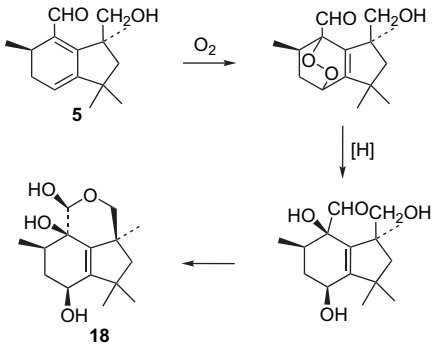
The singlet at δ 4.6, which was assigned to the proton H-10, the signals in the ^{13}C NMR at δ 72.2, C-1 and 138.1 and 146.4 corresponding to C9 and C5, respectively, were consistent with the proposed structure. The long-range experiments, which were carried out were consistent with the structure **18** proposed for this compound. The stereochemistry of the hydroxyl at C-1 was established in the same way as that of compound **16** by the downfield shift observed for the signal corresponding to methyl group on C-2. On the other hand the β configuration of hydroxyl at C-4 was assigned on the basis of the observed coupling constant and biogenetic considerations. Compound **18** has also been isolated in very small amounts in the experiment on the biotransformation of diisophorone, previously cited.[11] Its structure was not determined at that time.

The results obtained clearly show that [10-^2H,15-^2H]-botry-1(9)-4-diendiol (**12**) was not incorporated into the secobotryane derivative (**7**). However the high incorporation ratio observed in compounds **13–15** seems to indicate that, in addition to the previously proposed compound **9**,[7] the botrydiendiol (**12**) is the precursor of all the aromatic derivatives obtained from *B. cinerea*.[7,10]

Addition of oxygen to cisoid dienes to give peroxides has been reported in several fungi.[12] These epidioxides can rearrange or be reduced and several reports can be found in the literature.[12,13] The formation of compounds **16** and **17** represents two facets of the chemistry of the dienol **6**, Scheme 4. Compound **16** may be formed via the 1,4-epidioxide by a base-catalyzed cleavage whilst compounds **17/17b** are the result of a rearrangement. Compound **18** may be formed by the reductive cleavage of the 1,4-epidioxide formed from compound **5**, a putative intermediate to secobotryane **7**. If compound **18** was formed from **5** then it could explain why the former **18** had not incorporated deuterium, Schemes 5 and 6.



Scheme 4.



Scheme 5.

The new isolated compounds **16**–**18** shed further light on secondary metabolism of the fungus. Thus *B. cinerea* produce an interesting 1,4-dihydroxylation on the diene of the substrate leading to compounds **16**–**18**. From this point of view some modification on the degradative route of botrydial may be proposed, Scheme 6. Furthermore, the easy biotransformation and different biodegradative routes of botrydial seem to indicate that the toxins may not persist in the plant for a long time and can be metabolized by the fungus and the plant.

3. Experimental

3.1. General experimental procedures

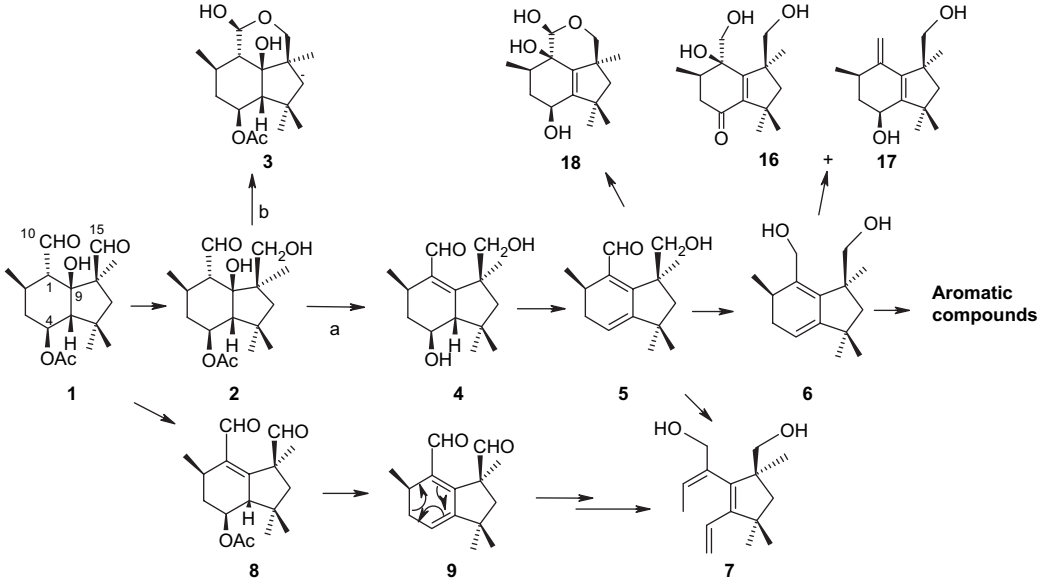
Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Mattson Genesis spectrophotometer, series FTIR (Fourier transform infrared). ^{1}H and ^{13}C NMR measurements were obtained on Varian Unity (^{1}H at 400 MHz, ^{13}C at 100 MHz) and Varian Unity 600 MHz (^{1}H at 600 MHz, ^{13}C at 150 MHz). Chemical shifts are quoted relative to TMS (Me_4Si) in $CDCl_3$. Mass spectra were recorded on Fisons MD800 and Finnigan MAT95 S instruments. High-performance liquid chromatography (HPLC) was performed with a Hitachi/Merck L-6270 apparatus equipped with an UV–vis detector (L4250) and a differential refractometer detector (RI-71). Thin-layer chromatography (TLC) was performed on Merck Kiesegel 60 F254, 0.2 mm thick (Catalog no. 1.05554.0001). Silica gel (Merck) was used for column chromatography. Chemicals were products of Fluka or Aldrich. Purification by means of HPLC was accomplished on a 25×1 cm Hibar 60 silica gel column. All solvents used were freshly distilled.

3.2. Organism

B. cinerea 2100 was obtained from the Colección Española de Cultivos Tipo (CECT), Facultad de Biología, Universidad de Valencia, Spain. Conidial stock suspensions of this strain were maintained viable in 80% glycerol at −40 °C.

3.3. Synthesis of [10-^{2}H,15-^{2}H]-botry-1(9)-4-diendiol 12

3.3.1. Botrydial 1. Sodium periodate (1.37 g, 6.4 mmol) was added to a solution of **10** (0.5 g, 1.6 mmol) in THF/H_2O (5:1, 10 mL). The solution was stirred for 12 h at room temperature. Sodium iodate precipitated out of the solution during the reaction and then it was filtered off. The mixture was concentrated in vacuo to remove THF. The resulting aqueous solution was extracted with EtOAc



Scheme 6.

(3×5 mL). The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo, and the crude extract was subjected to column chromatography to afford botrydial **1** (0.30 g, 60%), which was identified by its NMR spectroscopic data.

3.3.2. Botrydienal 9. A solution of **1** (100 mg, 0.32 mmol) in AcOEt (10 mL) was treated with 6% aqueous oxalic acid solution (15 mL) and refluxed for 12 h. The mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with AcOEt (×3). The organic layers were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed, and the mixture obtained was purified by means of normal-phase HPLC to afford **8**[2,5] (14 mg, 0.048 mmol, 15%), botrydienal **9**[2,9] (48.3 mg, 0.21 mmol, 65%) and aromatic derivative **11**[2,9] (7.4 mg, 0.032 mmol, 10%).

3.3.3. [10-^2H,15-^2H]-Botry-1(9)-4-diendiol 12. $NaBD_4$ (36.1 mg, 0.86 mmol) (≥99 at. % D, purchased from the Fluka Chemical Company) was added to a solution of **9** (50 mg, 0.22 mmol) in methanol (10 mL), and the resulting solution was stirred for 15 min at room temperature. The mixture was poured onto ice, acidified with 2 N HCl (15 mL), and stirred for 10 min. The solution was diluted with H_2O (45 mL) and extracted with $CHCl_3$ (30 mL, ×3). The solvent was evaporated and the crude extract chromatographed to yield **12** (50.3 mg, 0.21 mmol, 96%, HREIMS (*m/z*): 238.1908 (calcd $C_{15}H_{22}O_2D_2$, 238.1901)).

3.4. Biotransformation by *B. cinerea*

A suspension (4.2 mL) of *B. cinerea* conidia (2.48×10^6 conidia/mL) was inoculated at 25 °C and 250 rpm in shake cultures in 12 Erlenmeyer flasks (500 mL) containing 200 ml of Czapek–Dox medium: glucose (50 g), yeast extract (1 g), KH_2PO_4 (5 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g) and $FeSO_4$ (10 mg) per litre of distilled water. Before the inoculation, the pH medium was carefully adjusted to 7.0 with aqueous NaOH. After 72 h, the mycelium was transferred, into 10 flasks (500 mL) containing 200 mL of Czapek–Dox medium (without glucose) and the substrate **12** (90 ppm). The additional two flasks were used as control. After three days, the mycelium was filtered. The pH was measured (control: 6.09 pH; inoculated mediums broth: 6.03 pH). The broth (2 L) was saturated with sodium chloride and extracted four times with ethyl acetate. Extracts were dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue (203 mg) was purified by chromatography on a Si gel column and then by HPLC affording dehydrobotrydienol (**13**; 1.8 mg), 11-hydroxydehydrobotrydienol (**14**; 120 mg) and 12-hydroxydehydrobotrydienol (**15**; 3.5 mg), novel deuteriated compounds 1-hydroxy-4-oxo-[10-^{2}H, 15-^{2}H]-botry-5(9)-endiol (**16**; 0.3 mg) and botry-[1(10),5(9)-^2H]-dien-[15-^2H]-ol (**17**; 4 mg). Their structures were established on the basis of their NMR spectroscopy: ^{1}H, ^{2}H and ^{13}C HSQC, HMBC.

3.5. Biotransformation by *B. cinerea* on buffered medium

Twelve Erlenmeyer flasks (500 mL) were filled with 200 mL of Czapek–Dox medium as cited above. The pH was adjusted to 7.0 with aqueous NaOH, and the flasks were inoculated with a suspension (2.3 mL) of *B. cinerea* conidia (8×10^6 conidia/mL). The flasks were incubated at 25 °C for three days and stirred at 250 rpm; the mycelium was then filtered and transferred into 10 flasks (500 mL) containing 200 mL of medium, which consisted of equal volumes of sterile phosphate buffer (pH 7 with phosphate buffer 0.4 M) and Czapek–Dox (as above but without glucose) and 90 ppm of the substrate botry-4,9-dien-[15,10-^2H]-ol (**12**) per flask. The remaining two flasks were used as control. The broth and the mycelia were separated further by filtration after three days. The pH was measured (control: 6.58 pH units; inoculated medium broth: 6.51 pH units) and the broth was saturated with NaCl, and extracted four times with ethyl acetate. The extract was dried over anhydrous sodium sulfate, and the solvent was then evaporated under vacuum. Fractionation of the extract (210 mg) was carried out by means of column chromatography on silica gel (SiCC), eluting with hexane/ethyl acetate (80:20). Final purification was carried out by means of semi-preparative HPLC to afford deuteriated known compounds dehydrobotrydienol (**13**; 3 mg), 11-hydroxydehydrobotrydienol (**14**; 130 mg) and 12-hydroxydehydrobotrydienol (**15**; 1.1 mg), unknown deuteriated compounds, 1-hydroxy-4-oxo-[10-^{2}H, 15-^{2}H]-botry-5(9)-endiol (**16**; 0.6 mg) and botry-[1(10),5(9)-^2H]-dien-[15-^2H]-ol (**17**; 1.5 mg) and a new, undeuteriated, metabolite compound 1β,4β-dihydroxy-5(9)-ene dihydrobotrydial (**18**; 0.7 mg). Their structures were established on the basis of their one-dimensional and two-dimensional NMR analyses (^{1}H, ^{2}H, ^{13}C, HSQC and HMBC).

3.5.1. 1-Hydroxy-4-oxo-[10-^2H,15-^2H]-botry-5(9)-endiol 16. Colorless oil, IR (film): ν_{max} 3375, 2930, 1660, 1355, 1059, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.09 (3H, d, J_{11-2}=6.2 Hz, H-11), 1.27* (3H, s, H-13), 1.28* (3H, s, H-12), 1.38 (3H, s, H-14), 1.51 (1H, d, $J_{7\alpha-7\beta}$=12.9 Hz, H-7α), 1.76 (1H, d, $J_{7\beta-7a}$=12.9 Hz, H-7β), 2.39 (3H, m, H-2, H-3β, H-3α), 3.72–3.74 (2H, m, H10, H10′), 3.53 (1H, s, H-15), 3.76 (1H, s, H-15′). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm): 14.6 (q, C-11), 26.1 (q, C-14), 28.2* (q, C-13), 29.8* (q, C-12), 42.0 (d, C-2), 42.4 (t, C-6), 44.3 (s, C-3), 52.0 (s, C-7), 52.4 (t, C-8), 64.4 (dt$^{\#}$, C-10), 68.4 (dt$^{\#}$, C-15), 75.6 (s, C-1), 145.0 (s, C-5), 169.1 (s, C-9), 197 (s, C-4). *: Interchangeable; $^{\#}$: coupling ^{13}C–^{1}H (d) and ^{13}C–^{2}H (t). EIMS (*m/z*): 270 [M$^+$] (3%), 252 [M$^+$–H_2O] (0.2%), 238 [M$^+$–CDHOH] (55%), 220 [M$^+$–H_2O–CDHOH] (100%). HREIMS (*m/z*): 270.1772 (calcd $C_{15}D_2H_{22}O_4$, 270.1800). Deuterium percentage: 44%.

3.5.2. Botry-[1(10),5(9)-^2H]-dien-[15-^2H]-ol 17. Colorless oil, $[\alpha]_D^{25}$ +12.20 (*c* 1.5, ethyl acetate); IR (film): ν_{max} 3386, 2950, 2933, 1459, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.12 (3H, d, J_{11-2}=6.7 Hz, H-11), 1.25 (3H, s, H-12), 1.17 (3H, s, H-13), 1.29 (3H, s, H-14), 1.54 (1H, d, $J_{7\alpha-7\beta}$=13.0 Hz, H-7α), 2.09 (1H, d, $J_{7\beta-7a}$=13.0 Hz, H-7β), 1.65 (1H, ddd, $J_{3\alpha-3\beta}$=13.2 Hz, $J_{3\alpha-2}$=10 Hz, $J_{3\alpha-4}$=4.8 Hz, H-3α), 1.8 (1H, ddd, $J_{3\beta-3\alpha}$=13.2 Hz, $J_{3\beta-4}$=4.8 Hz, $J_{3\beta-4}$=3.9 Hz, H-3β), 2.6 (1H, m, H-2), 3.41 (1H, br s, H-15), 3.8 (1H, br s, H-15′), 4.39 (1H, dd, $J_{4-3\alpha}$=4.8 Hz, $J_{4-3\beta}$=4.8 Hz, H-4), 4.9 (1H, s, H-10), 5.11 (1H, s, 10). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.2 (q, C-11), 19.3 (q, C-14), 31.15 (q, C-12), 28.39 (q, C-13), 33.12 (d, C-2), 41.62 (t, C-3), 50.67

(s, C-8), 43.43 (s, C-6), 53.25 (t, C-7), 63.59 (d, C-4), 137.6 (s, C-1), 69.30 (C-15), 145.9 (s, C-9), 150.7 (s, C-5), 107.3 (C-10). EIMS (*m/z*): 238 [M^+] (79%), 223 [M^+–Me] (7%), 205 [M^+–H_2O–Me] (79%), 188 [M^+–H_2O–CDHOH] (100%). HREIMS (*m/z*): 238.1895 (calcd $C_{15}D_2H_{22}O_2$, 238.1902). Deuterium percentage: 79%.

3.5.3. Botry-1(10),5(9)-dien-15-ol 17b. Colorless oil, $[\alpha]_D^{25}$ +12.14 (*c* 1.4, ethyl acetate); IR (film): ν_{max} 3386, 2958, 2930, 1459, 1375 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.12 (3H, d, J_{11-2}=6.7 Hz, H-11), 1.25 (3H, s, H-12), 1.16 (3H, s, H-13), 1.29 (3H, s, H-14), 1,53 (1H, d, $J_{7\alpha-7\beta}$=13.0 Hz, H-7α), 2.08 (1H, d, $J_{7\beta-7a}$=13.0 Hz, H-7β), 1.65 (1H, ddd, $J_{3\alpha-3\beta}$=13.2 Hz, $J_{3\alpha-2}$=10 Hz, $J_{3\alpha-4}$= 4.8 Hz, H-3α), 1.8 (1H, ddd, $J_{3\beta-3\alpha}$=13.2 Hz, $J_{3\beta-4}$= 4.8 Hz, $J_{3\beta-4}$=3.9 Hz, H-3β), 2.6 (1H, m, H-2), 3.43 (1H, d, $J_{15-15'}$=11.1 Hz, H-15), 3.8 (1H, d, $J_{15'-15}$=11.1 Hz, H-15′), 4.39 (1H, dd, $J_{4-3\alpha}$=4.8 Hz, $J_{4-3\beta}$=4.8 Hz, H-4), 4.95 (1H, s, H-10), 5.13 (1H, s, 10). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.2 (q, C-11), 19.13 (q, C-14), 31.15 (q, C-12), 28.39 (q, C-13), 33.12 (d, C-2), 41.62 (t, C-3), 50.67 (s, C-8), 43.43 (s, C-6), 53.25 (t, C-7), 63.59 (d, C-4), 137.6 (s, C-1), 69.77 (t, C-15), 145.9 (s, C-9), 150.7 (s, C-5), 107.61 (t, C-10). EIMS (*m/z*): 237 [M^++1] (79%), 236 [M^+] (7%), 218 [M^+–H_2O]. HREIMS (*m/z*): 236.1735 (calcd $C_{15}H_{24}O_2$, 236.1731).

3.5.4. 1β,4β-Dihydroxy-5(9)-ene dihydrobotrydial 18. Colorless oil, $[\alpha]_D^{25}$ −55.2 (*c* 0.096, $CHCl_3$); IR (film): ν_{max} 3410, 2954, 2924, 1639, 1455, 1035, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.1 (3H, d, J_{11-2}= 7.5 Hz, H-11), 1.2 (3H, s, H-12), 1.24 (3H, s, H-13), 1.4 (3H, s, H-14), 1,6 (2H, d, $J_{7\alpha-7\beta}$=1.2 Hz, H-7), 1.7 (1H, ddd, $J_{3\alpha-3\beta}$=14.5 Hz, $J_{3\alpha-2}$=2.8 Hz, $J_{3\alpha-4}$=1.8 Hz, H-3α), 2.01 (1H, m, H-2), 2.15 (1H, dt, $J_{3\beta-3\alpha}$=14.5 Hz, $J_{3\beta-2}$= $J_{3\beta-4}$=4.7 Hz, H-3β), 3.2 (1H, d, $J_{15\alpha-15\beta}$=10.7 Hz, H-15α), 3.8 (1H, d, $J_{15\beta-15\alpha}$=10.7 Hz, H-15β), 4.3 (1H, dd, $J_{4-3\alpha}$=1.6 Hz, $J_{4-3\beta}$=4.8 Hz, H-4), 4.6 (1H, s, H-10). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.3 (q, C-11), 27.0 (q, C-14), 28.07 (q, C-12), 30.7 (q, C-13), 33.9 (d, C-2), 35.9 (t, C-3), 43.7 (s, C-8), 46.2 (s, C-6), 51.7 (t, C-7), 62.8 (d, C-4), 72.2 (s, C-1), 77.2 (t, C-15), 97.3 (s, C-10), 138.1 (s, C-9), 146.4 (s, C-5). EIMS (*m/z*): 268 [M^+] (0.2%), 250 [M^+–H_2O] (11%), 239 (27%), 204 (49%), 191 (100%), 189 (52%), 165 (37%), 161 (32%), 148 (44%), 133 (51%), 91 (42%). HREIMS (*m/z*): 250.1579 (–H_2O) (calcd $C_{15}H_{22}O_3$, 250.1569).

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References and notes

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