The metabolism of the sesquiterpenoid 12-nor-8α-presilphiperfolan-9β-ol by the fungus *Botrytis cinerea*

Isidro G. Collado[b]*, Athina Deligeorgopoulo[a], James R. Hanson[a]*, Peter B. Hitchcock[a] and Antonio J. Macias-Sánchez[b]

[a]*Department of Chemistry, University of Sussex, Brighton, Sussex BN1 9QJ, UK*

[b]*Departamento de Quimica Organica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain*

12-Nor-8α-presilphiperfolan-9β-ol has been prepared from caryophyllene oxide as a mimic of botryane biosynthetic intermediates and its metabolism by Botrytis cinerea has been studied.

Keywords: 12-Nor-8-presilphiperfolan-9-ol, biosynthesis, botryane, *Botrytis cinerea*

The biosynthesis of the phytotoxic sesquiterpenoid botryane [1,2] metabolites of the fungus, *Botrytis cinerea*, involves the cleavage of a tricyclic metabolite[3] such as **1** to form botrydial **2a** and dihydrobotrydial **2b**.[1,2] 12-Nor-8α-presilphiperfolan-9β-ol **8**[4] possesses a formal similarity to these tricyclic intermediates. Consequently it was of interest to see if this compound was metabolised along the biosynthetic pathway and whether it had any effect on the growth of the fungus.

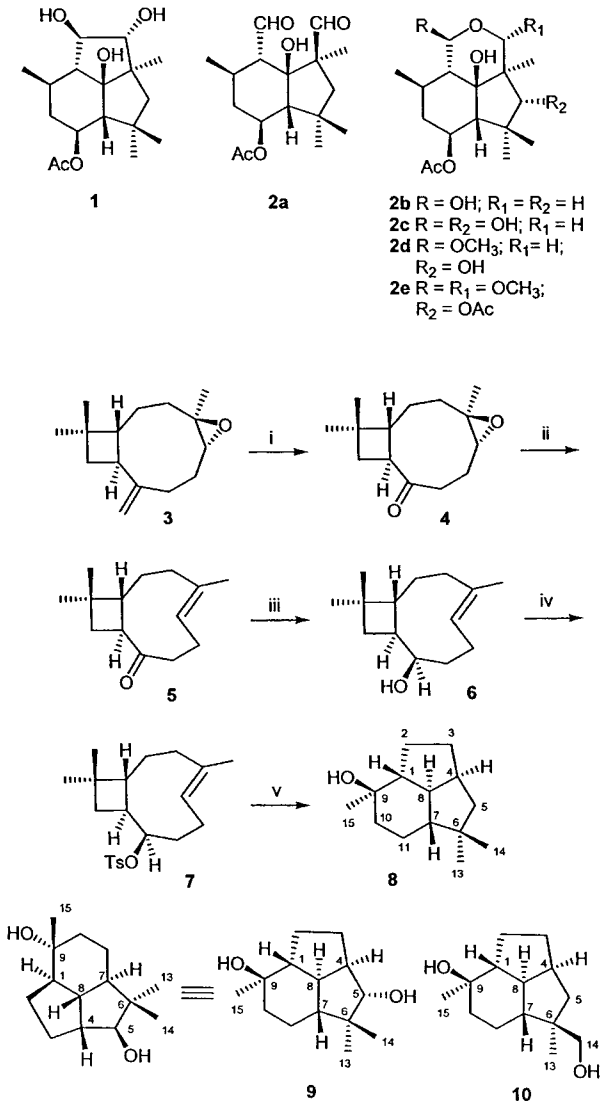
12-Nor-8α-presilphiperfolan-9β-ol **8** was obtained by a modification of the literature route[4] from caryophyllene oxide via kobusone (see Scheme 1). Kobusone **4**[5] was obtained from caryophyllene oxide **3** by oxidation with potassium permanganate, iron(II) sulfate and sodium dihydrogen phosphate. Deoxygenation of the epoxide with zinc gave the unsaturated ketone **5**[6] which was reduced with lithium aluminium hydride to the alcohol **6**. Solvolysis of the toluene-*p*-sulfonate **7** of this alcohol gave 12-nor-8α-presilphiperfolan-9β-ol 8.[4]

This compound possessed a fungistatic action against *B.cinerea* but only at quite high concentrations (see Table 1). The effect diminished with time suggesting that the compound was being metabolised.

12-Nor-8α-presilphiperfolan-9β-ol **8** was incubated with *B. cinerea* for 7 days. Two metabolites were isolated and separated by chromatography. The first metabolite was 12-nor-8α-presilphiperfolan-5α,9β-diol **9**. The ^{1}H and ^{13}C NMR spectra showed that a new secondary alcohol was present (δH 3.54, dd, *J*=1.1 and 7.7 Hz; δC 90.3). The signal assigned to C-5 in the starting material was missing whilst the signal assigned to C-4 showed a significant downfield shift (Δδ 9.1) (see Table 2). The magnitude of the H-4:H-5 coupling constant and the presence of a long-range coupling on the H-5 resonance suggested that the alcohol had the 5α stereochemistry. The structure **9** (1R,4R,5S,7R,8R,9R)-6,6,9-trimethyltricyclo[6.2.1.0^{5,11}]undecane-5,9-diol) of the metabolite was confirmed by X-ray crystallography (see Fig. 1). The ^{1}H NMR spectrum of the second metabolite lacked a methyl group signal shown in the starting material which had been replaced by a primary alcohol resonance [δ_H 3.49 (2H, s); δ_C 71.9] Irradiation of the 8-H signal at δ_H 1.84 gave nOe enhancements to the methyl group signals at δ_H 1.27 and 0.91 and to the 4-H signal at δ_H 2.20 showing that these protons were on the same face of the molecule. Hence the hydroxymethyl group was on the opposite (β) face. Both C-5 and C-7 show a γ-gauche shielding in accord with this stereochemistry. This leads to the assignment of the structure as 12-nor-8α-presilphiperfolan-9β,14-diol **10**.

These results suggest that compound **8**, despite its formal similarity to the botryane precursors, is not apparently being metabolised to any readily observable extent along the pathway leading to botrydial **2a**. Nevertheless compounds with the

* Correspondence. E-mail: j.r.hanson@susx.ac.uk



Scheme 1 (i) KMnO4, FeSO4, NaH2PO4; (ii) Zn; (iii) LiAlH4; (iv) TsCl, pyr., DMAP; (v) aq.butanone, pyr. 70˚C.

Table 1 Inhibition of *B.cinerea* by 12-nor-8α-presilphiperfolan-9β-ol

% Decrease in diameter of *B.cinerea substrate* vs Time Diameter of *B.cinerea* control		
Time (h)	100 ppm	200 ppm
24	33	66
48	40	60
96	22	33
144	24	48

Table 2 ^{13}C NMR data for compounds **8**, **9** and **10**

Carbon atom	Compound **8**	**9**	**10**
1	59.6	59.8	60.0
2	31.7	31.9	32.1
3	35.4	34.1	35.7
4	35.7	44.8	35.8
5	51.2	90.5	46.0
6	47.2	49.9	52.9
7	58.2	55.1	54.5
8	56.3	52.7	56.4
9	76.1	76.2	76.5
10	45.5	45.0	45.8
11	26.8	27.5	28.2
13	20.2	14.9	71.9
14	21.2	22.1	16.1
15	27.1	25.2	22.1

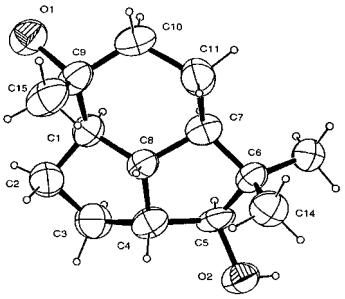


Fig. 1 X-ray crystal structure of compound **9**.

botryane skeleton bearing an oxygen atom adjacent to the gem-dimethyl group (**2c**–**e**) have been isolated from the ascomycete, *Hymenoscyphus epiphyllus*.[7] However, compound **8** does show some fungistatic activity against *Botrytis cinerea.*

Experimental

General experimental details: Silica for chromatography was Merck 9385. Light petroleum refers to the fraction b.p. 60–80°C. ^{1}H and ^{13}C NMR spectra were determined at 300 and 75 MHz respectively for solutions in deuteriochloroform. IR spectra were determined as nujol mulls. Mass spectra were determined on a Fisons Autospec mass spectrometer. Extracts were dried over sodium sulfate.

Preparation of kobusone from caryophyllene oxide: Potassium permanganate (5 g), iron(II) sulfate (2.5 g) and sodium dihydrogen orthophosphate (1.25 g) were ground together to a fine powder in a pestle and mortar. Water (0.25 cm^3) was added and the mixture was then added to a solution of caryophyllene oxide **3** (1 g) in dichloromethane (30 cm^3) and *t*-butanol (1.25 cm^3). The mixture was stirred at room temperature for 24 h. It was then filtered through a Celite pad. The pad was washed with dichloromethane and the washings were combined with the dichloromethane solution. The dichloromethane solution was washed with aqueous sodium hydrogen carbonate, water and dried. The solvent was evaporated and the residue chromatographed on silica. Elution with 5% ethyl acetate:light petroleum gave kobusone **4** (0.45 g) which was identified by its IR and ^{1}H NMR spectra.[5]

Deoxygenation of kobusone: Zinc powder (100 g) was ground in a pestle and mortar for 10 min. with 10% hydrochloric acid (12 cm^3). The acid was decanted and the solid was washed with acetone (× 3), ether (× 3) and then with dry ethanol (× 3). The zinc was immediately added to a solution of kobusone **4** (2.7 g) in dry ethanol (190 cm^3) which was then heated under reflux for 36 h. The solution was filtered, concentrated and diluted with water (125 cm^3). The solution was extracted with ether and the extract was washed with brine and dried. The solvent was evaporated to give (E)-13-norcaryophyll-4-en-8-one **5**[6] (1.2 g) as an oil, (lit.,[6] m.p. 32°C), ν_{max}/cm^{-1} 1696, 1632; δ_H 0.97 (3H, s,), 1.01 (3H, s), 1.75 (3H, s), 2.65 (2H, m), 2.80 (1H, m), 5.23 (1H, dd, *J*=6.5,7,7 Hz).

Reduction with lithium aluminium hydride: (E)-13-Norcaryophyll-4-en-8-one (1.1 g) in dry ether (18 cm^3) was treated with lithium aluminium hydride (220 mg) at room temperature for 30 min. Ethyl acetate was added to destroy the excess reagent. The organic phase was washed with water, dried and the solvent evaporated to give (E)-13-norcaryophyll-4-en-8β-ol **6** (1 g) as an oil (lit.,[4] oil), ν_{max}/cm^{-1} 3401, 1635; δ_H 0.93 (3H, s), 0.94 (3H, s), 1.60 (3H, s), 3.62 (1H, br.s.), 5.35 (1H, br.s).

The toluene-*p*-sulfonate **7**, prepared with toluene-*p*-sulfonyl chloride in pyridine containing dimethylaminopyridine, had m.p.88–90°C, (lit.,[4] 88-89°C), ν_{max}/cm^{-1} 1637, 1596; δ_H 0.85 (3H, s), 0.86 (3H, s), 1.57 (3H, s), 2.45 (3H, s), 4.51 (1H, br.d, *J*=7.6 Hz), 5.34 (1H, t, *J*=7,4 Hz), 7.34 (2H, d, *J*=8.3 Hz), 7.84 (2H, d, *J*=8.3 Hz).

Preparation of 12-nor-8α-silphiperfolan-9β-ol: The above toluene-*p*-sulfonate **7** (700 mg) was dissolved in 60% aqueous butanone (125 cm^3) and pyridine (0.84 cm^3) and heated at 80°C for 2 days. The mixture was cooled to 0°C and diluted with 1:1 hexane:ether (70 cm^3). The organic layer was washed with aqueous copper sulfate, water, brine and dried. The solvent was evaporated and the residue was chromatographed on silica. Elution with 15% ethyl acetate:light petroleum gave 12-nor-8α-presilphiperfolan-9β-ol **8** (210 mg) which crystallised from ethyl acetate as needles, m.p. 106–108°C (lit.,[4] m.p.107–109°C), ν_{max}/cm^{-1} 3364; δ_H 0.81 (3H, s,), 0.98 (3H, s,) 1.24 (3H, s).

Incubation of 12-nor-8α-presilphiperfolan-9β-ol with B.cinerea: *Botrytis cinerea* (UCA 992) was grown in shake culture at 25°C in 250 cm^3 conical flasks in a medium (70 cm^3 per flask) comprising (per litre), glucose (40 g), yeast extract (1 g), potassium dihydrogen phosphate (5 g), sodium nitrate (2 g), magnesium sulfate (0.5 g), iron(II) sulfate (10 mg) and zinc sulfate (5 mg) for 3 d. 12-Nor-8α-presilphiperfolan-9β-ol **8** (150 mg) in ethanol (12 cm^3) was evenly distributed between 12 flasks and the fermentation was continued for a further 7 days. The mycelium was filtered and washed with ethyl acetate.The broth was acidified to pH 2 and then extracted with ethyl acetate. The combined ethyl acetate extracts were washed with aqueous sodium hydrogen carbonate, water and dried. The solvent was evaporated and the residue was chromatographed on silica. Elution with 15% ethyl acetate:light petroleum gave 12-nor-8α-presilphiperfolan-5α,9β-diol **9** (10 mg), m.p. 161–163°C, (Found: M$^+$ 224.1789. $C_{14}H_{24}O_2$ requires 224.1776), ν_{max}/cm^{-1} 3328; δ_H 0.77 (3H, s), 0.97 (3H, s,), 1.24 (3H, s) 3.54 (1H, dd, *J*=1.1 and 7.7 Hz). Further elution with 20% ethyl acetate:light petroleum gave 12-nor-8α-presilphiperfolan-9β,14-diol **10** (15 mg) which crystallised from ethyl acetate as needles, m.p. 158–159°C, (Found: M$^+$ 224.1788. $C_{14}H_{24}O_2$ requires 224.1776), ν_{max}/cm^{-1} 3335; δ_H 0.91 (3H, s), 1.27 (3H, s),1.65 (1H, dd, *J*=4.2 and 11.6 Hz), 1.84 (1H, dd, *J*=11.4 and 12.6 Hz), 2.20 (1H, td, *J*=7.6 and 12.4 Hz,), 3.49 (2H, br.s,),

Inhibition of B.cinerea[8] Malt agar (1 l) was prepared from glucose (30 g), malt extract (30 g), peptone (3 g) and agar (15 g) and sterilised. The plates each contained 19 cm^3 medium. 12-Nor-8α-presilphiperfolan-9β-ol in ethanol (1 cm^3) was added to each plate to give a final concentration of 100 and 200 ppm. The ethanol content was identical in the control A 13 mm disc of actively growing *B.cinerea* was placed in the centre of each plate. The plates were incubated at 25°C and the diameter of the growing culture was measured for 4 days (see Table 1).

X-Ray crystal data and structure determination: 12-Nor-8α-presilphiperfolan-5α,9β-diol **9**, C14H24O2, *Mr* 224.33, rhombohedral, space group R3 (No146), *a* = 25.524(8), *b* = 25.524(8), *c* = 6.734(2)Å, α = β = 90°, γ = 120°, *V* = 3799(2)Å3, *Z* = 9, D_{calc} 0.88 gcm^{-3}, μ = 0.06 mm^{-1}, F(000) = 1116, crystal size 0.10 × 0.05 × 0.02 mm^3. A total of 5145 reflections were collected on a KappaCCD diffractometer for 3.89 < θ < 21.96° and –24 ≤ *h* ≥ 26, –26 ≤ *k* ≥ 26, –6 ≤ *l* ≥ 7. There were 1975 independent reflections and 1242 reflections with I > 2σ(I) were used in the refinement. The structure was solved by direct methods and refined using SHELXL-97. The final R indices were [*I* > 2 σ (I)] R_1 = 0.115, wR_2 = 0.292 and (all data) R_1 = 0.167, wR_2 = 0.329. The largest difference peak and hole was 0.48 and –0.25eÅ^{-3}. Repeated crystallisation always resulted in small inter-grown needles. Data were finally collected on a small fragment cut from a cluster and consequently the data was weak. The crystal structure showed large channels parallel to 'c' which may have contained solvent but for which the structure determination showed only 'noise'. Nevertheless, taken with the other spectroscopic data, the structure was adequate for locating the hydroxyl group at C-5 and to assign the stereochemistry as 5S based on the known absolute stereochemistry of the precursor.

The X-ray data has been deposited at the Cambridge Crystallographic Data Centre as CCDC 235188.

Received 6 April 2004; accepted 8 June 2004
Paper 04/2442

References

1 J.R. Hanson, *Pure Appl. Chem*., 1981, **53**, 1155.
2 R. Duran-Patron, A.J. Colmenares, A. Montes, J.R. Hanson, R. Hernandez-Galan and I.G. Collado, *Tetrahedron*, 2003, **59**, 6267 and refs therein.
3 I.G. Collado, R. Hernandez-Galan, R. Duran-Patron and J.M. Cantoral, *Phytochemistry*, 1995, **38**, 647.
4 S. Shankar and R.M. Coates, *J. Org. Chem.*,1998, **63**, 9177.
5 H. Hikino, K. Aota and T. Takemoto, *Chem. Pharm. Bull*.,1969, **17**, 1390.
6 R. Kaiser and D. Lamparsky, *Helv. Chim. Acta*, 1976, **59**,1803.
7 E. Thines, H. Anke, W. Steglich and O. Sterner, *Zeitschrift Naturforschung, C: Biosci.*, 1997, **52**, 413.
8 S. Patil, S. Kulkarni and R.K. Hegde, *Pesticides*, 1986, 30.