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Transformations of hispanolone. Novel Michael adducts with in planta activity against rice blast

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Abstract—Two novel Michael adducts 9α-cyano-15,16-epoxy-7β-hydroxylabda-13(16),14-dien-6-one (**2**) and 9α-cyano-15,16-epoxy-7-hydroxylabda-7,13(16),14-trien-6-one (**3**) and the reduction product of **2**, 9α-cyano-15,16-epoxy-6β,7β-dihydroxylabda-13(16),14-diene (**4**), were synthesized from the naturally occurring labdane diterpene hispanolone (**1**). Compounds **2**–**4** exhibited in planta activity against the pathogenic rice blast fungus *Magnaporthe grisea*.

1. Introduction

The filamentous ascomycete fungus *Magnaporthe grisea* (Herbert) Barr (anamorph *Pyricularia grisea*=*P. oryzae*), the causal agent of the leaf spot disease known as rice blast, is a pathogen of over fifty different grass species including economically important crops such as rice, wheat, and barley.[1] Rice blast is a serious disease of cultivated rice in most rice growing nations (ca. 85 countries worldwide) and losses in excess of 50% of the global annual crop yield can be attributed directly to this pathogenic fungus.[2] In mature rice plants the fungus prevents maturation of the rice grains by attacking the panicle (the inflorescence that holds the rice grain). Infection of the panicle is known as 'neck blast' and can ultimately lead to total crop loss.[1,2] As part of an ongoing screening programme of both natural products and their semi-synthetic derivatives for in planta inhibition of pathogenic fungal infection we have identified two novel Michael adducts, 9α-cyano-15,16-epoxy-7β-hydroxylabda-13(16),14-dien-6-one (**2**) and 9α-cyano-15,16-epoxy-7-hydroxylabda-7,13(16),14-trien-6-one (**3**), of the naturally occurring labdane diterpene hispanolone (**1**), which exhibited in planta inhibition of rice blast. 9α-Cyano-15,16-epoxy-6β,7β-dihydroxylabda-13(16),14-diene (**4**), the reduction product of **2**, was also active against rice blast. The synthesis of **2**–**4** from **1** is outlined in Scheme 1.

2. Results and discussion

Hispanolone was first isolated from *Ballota hispanica*[3] and has subsequently been isolated from other Lamiaceae sp.[4–7] including the endemic southern African medicinal plant *B. africana*.[8] Hispanolone is an abundant constituent of this latter plant and can be readily dehydrated to afford hispanone (**5**). Hispanolone and hispanone have proved to be useful precursors for the semi-syntheses of a number of related diterpenes[9–12] and we recently, converted **1** into 6β-hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one (**6**), the enantiomer of a diterpene metabolite previously isolated from *B. aucheri*.[13] It was en route to **6** that we serendipitously prepared **2** and the minor compounds **3**, **7** and **8** (Scheme 1).

An acetone extract of air-dried *B. africana* leaves was concentrated in vacuo and the resultant solution adsorbed onto HP-20 polystyrene resin. Subsequent gradient elution (acetone/water) of this resin afforded **1** in a substantially enhanced yield (1.5%) compared to that previously obtained from this plant (0.8%).[8] Facile dehydration of **1** with iodine in refluxing anhydrous benzene gave **5** as pale yellow needles.[13] The treatment of **5** with manganic acetate [$Mn(OAc)_3$] in refluxing anhydrous benzene[13,14] gave a

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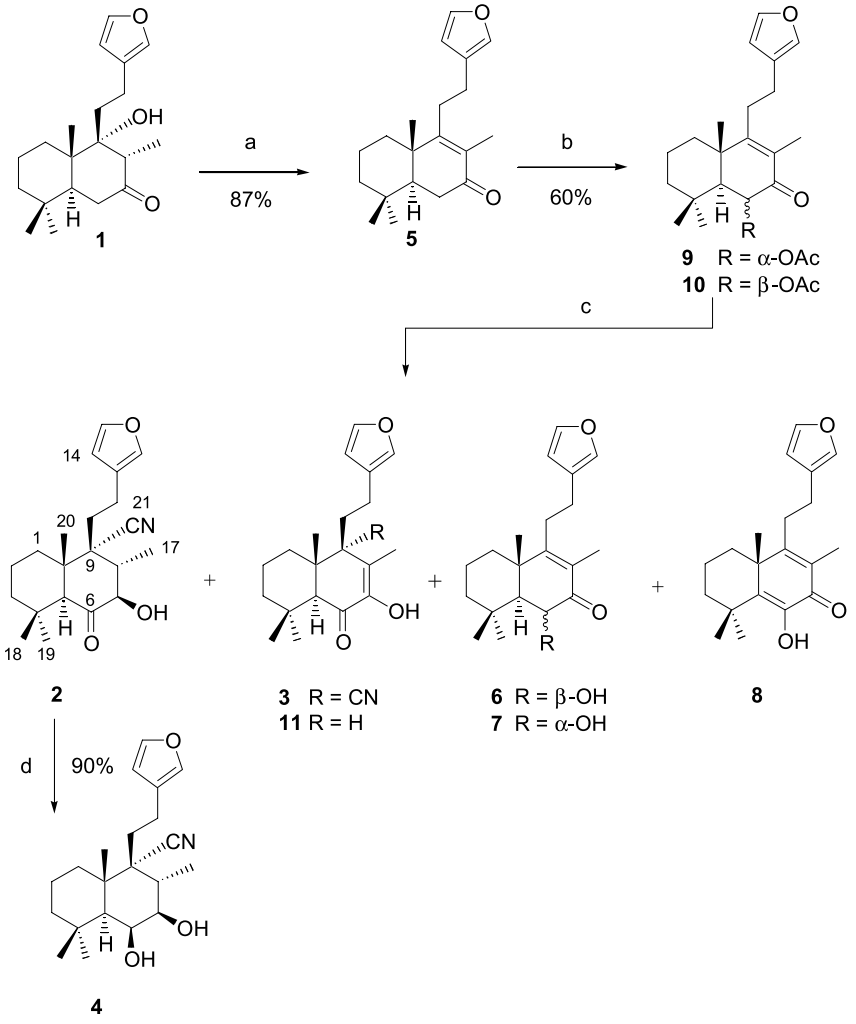


Scheme 1. (a) I_2, C_6H_6, reflux; (b) $Mn(OAc)_3$, C_6H_6, reflux; (c) KCN, EtOH (95%); (d) LAH, THF, reflux.

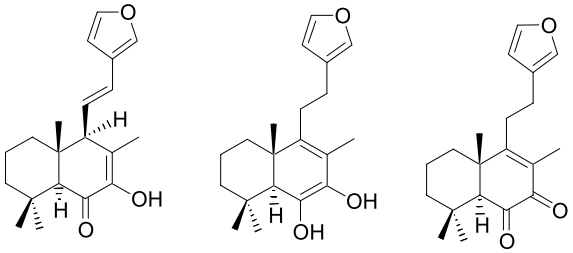
5:2 ratio of 6α-acetoxy- and 6β-acetoxyhispanone (**9** and **10**, respectively), in 60% overall isolated yield. Unfortunately, we were unable to improve the yields of **9** and **10** using the recently published method of Demir et al., in which the addition of up to 10% acetic acid to the reaction mixture was reported to enhance the manganic acetate mediated α′-acetoxylation of α,β-unsaturated enones in benzene.[15] Nonetheless, our preparation of **9** and **10** from **5** constitutes a significant improvement in the yield (19%) of these compounds achieved by Rodríguez and co-workers for this transformation.[9]

Our initial attempts at converting **10** to the *B. aucheri* metabolite (**6**), via saponification with ethanolic KOH, did not yield the desired product and instead gave **7** (4%) and the two diosphenols **8** (26%) and **11** (30%). We have previously isolated **8** as a minor product from Vedejs' oxidation of hispanone.[13] The ^{1}H NMR data and optical rotation of **11** ($[\alpha]_D+2$) were compatible with those reported for dihydro-7-hydroxyhedychenone ($[\alpha]_D+1$).[16] Compound **11** is the Δ^{11} hydrogenation product of 7-hydroxyhedychenone (**12**), a furanolabdane diterpene previously isolated from the rhizomes of *Hedychium*

Table 1. ^{1}H (400 MHz, $CDCl_3$), ^{13}C (100 MHz, $CDCl_3$) data for compound **11**

Carbon	δ_C ppm (mult.)	δ_H ppm (mult., int., *J*/Hz)
1	38.4 (t)	1.15 (1H, m), 1.81 (1H, d, 12.9)
2	18.0 (t)	1.48 (2H, m)
3	43.0 (t)	1.18 (1H, m), 1.40 (1H, d, 15)
4	32.5 (s)	
5	62.4 (d)	2.10 (1H, s)
6	195.2 (s)	
7	143.9 (s)	
8	126.4 (s)	
9	53.9 (d)	2.17 (1H, br d, 6.9)
10	43.6 (s)	
11	28.2 (t)	1.68 (2H, m)
12	27.1 (t)	2.49 (1H, m)
		2.65 (1H, m)
13	124.5 (s)	
14	110.9 (d)	6.29 (1H, s)
15	143.0 (d)	7.37 (1H, s)
16	138.9 (d)	7.25 (1H, s)
17	13.58 (q)	1.92 (3H, s)
18	33.4 (q)	1.17 (3H, s)
19	21.5 (q)	1.13 (3H, s)
20	14.8 (q)	0.84 (3H, s)
7-OH		6.22 (1H, s)

spicatum.[16] Sharma et al.,[16] provided limited ^{1}H NMR data of **11** acquired at 60 MHz and the fully assigned ^{1}H and ^{13}C NMR data of **11**, prepared from the saponification of **10**, are accordingly presented in Table 1.



Interestingly, the saponification of the 6α-acetoxy epimer (**9**) with ethanolic KOH afforded the same three products in similar yields suggesting that the enediol (**13**) is a possible precursor of **7** and **11**. The formation of **8** is more difficult to rationalise as this transformation possibly involves initial oxidation of either **6** or **7** to give the diketone **14**. Although no diketone was evident amongst the reaction products an autooxidative transformation of either **6** or **7** to **14** is supported by evidence for the facile α-oxidation of ketones in aerated ethanolic KOH solutions.[17] Subsequent keto–enol tautomerism of **14** would afford the conjugated enol **8**.

The results of the unsuccessful KOH saponification of **9** and **10** prompted us to investigate other ester hydrolyses and we accordingly applied a milder KCN mediated hydrolysis procedure to **9** and **10** (Scheme 1).[18] Semi-preparative HPLC separation of the reaction products from this hydrolysis gave the unexpected Michael adduct (**2**) as the major product (45%) together with three minor products **3** (9%), **7** (1%) and **8** (2%). Both **9** and **10** gave the same product mixture, thus making prior separation of the epimers unnecessary.

The incorporation of a nitrile moiety into the diterpene skeleton of **2** was supported firstly by the molecular formula ($C_{21}H_{29}NO_3$) established from HRFABMS data and secondly from a nitrile stretching absorption (ν_{max} 2228 cm^{-1}) in the IR spectrum of **2**. The nitrile functionality was positioned at C-9 (δ_C 53.4) from the three bond HMBC correlations (Fig. 1) observed between neighbouring protons and the nitrile carbon (C-21, δ_C 120.3). HMBC data were similarly instrumental in confirming the 1,2-transposition of the carbonyl moiety,

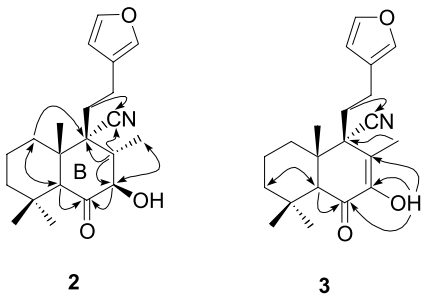


Figure 1. Key HMBC correlations used to establish the structure of ring B in compounds **2** and **3**.

originally at C-7 in **9** and **10**, to C-6 in **2** (Fig. 1). The singlet attributed to H-5 (δ_H 2.73) together with the doublet and the doublet of quartets assigned, respectively, to the H-7 (δ_H 3.92) and H-8 (δ_H 1.87) resonances in the ^{1}H NMR spectrum of **2** further supported the substitution pattern proposed for ring B. The *trans* diaxial relationship between H-7 and H-8 was confirmed from the coupling constant ($J_{7,8}$ = 10.8 Hz) and a NOESY correlation between the α-axial proton H-7 and both H-5 and the protons of the α-equatorial methyl substituent at C-8. The α-axial orientation of the nitrile moiety at C-9 was proposed from NOESY correlations between the C-11 methylene protons (δ_H 1.54 and 1.98) and both the H-8 methine proton and the protons of the angular methyl group at C-10 (δ_H 0.89). The ^{1}H and ^{13}C NMR data of **2**, assigned from exhaustive 2D NMR experiments, are presented in Table 2.

Table 2. ^{1}H (400 MHz, $CDCl_3$), ^{13}C (100 MHz, $CDCl_3$) data for compounds **2** and **3**

	2		**3**	
Carbon	δ_C ppm (mult.)	δ_H ppm (int., mult., *J*/Hz)	δ_C ppm (mult.)	δ_H ppm (int., mult., *J*/Hz)
1	35.2 (t)	1.75 (2H, br t, 6.2)	35.1 (t)	1.80 (2H, m)
2	18.1 (t)	1.61 (2H, m)	17.8 (t)	1.60 (2H, m)
3	41.6 (t)	1.28 (1H, m), 1.38 (1H, m)	41.9 (t)	1.32 (1H, m) 1.45 (1H, m)
4	32.7 (s)		32.8 (s)	
5	59.7 (d)	2.73 (1H, s)	58.6 (d)	2.75 (1H, s)
6	209.0 (s)		193.6 (s)	
7	77.3 (s)	3.92 (1H, br d, 10.8)	145.0 (s)	
8	47.1 (d)	1.87 (1H, dq, 10.8, 6.5)	120.0 (s)	
9	53.4 (s)		46.0 (s)	
10	47.0 (s)		52.5 (s)	
11	31.6 (t)	1.54 (1H, m), 1.98 (1H, m)	31.6 (t)	1.85 (1H, m), 1.99 (1H, m)
12	24.6 (t)	2.70 (2H, m)	24.7 (t)	2.82 (m)
13	123.6 (s)		123.6 (s)	
14	110.5 (d)	6.27 (1H, d, 0.9)	110.5 (d)	6.29 (1H, d, 0.9)
15	143.2 (d)	7.36 (1H, t, 1.6)	143.3 (d)	7.38 (1H, t, 1.6)
16	138.8 (d)	7.26 (1H, br s)	138.8 (d)	7.28 (1H, br s)
17	14.7 (q)	1.42 (3H, d, 6.5)	12.6 (q)	2.01 (3H, s)
18	32.3 (q)	1.02 (3H, s)	33.4 (q)	1.24 (3H, s)
19	22.1 (q)	1.30 (3H, s)	21.5 (q)	1.16 (3H, s)
20	15.8 (q)	0.89 (3H, s)	15.7 (q)	1.00 (3H, s)
21	120.3 (s)		119.5 (s)	
7-OH		3.71 (1H, br d, 3.0)		6.46 (br s)

The mechanism for the formation of **2** is of interest and appears to involve an initial Michael addition of a cyanide nucleophile with re facial selectivity to the α,β-unsaturated ketone in either **9** or **10**, followed by a 1,2-carbonyl transposition through a classic Lobry de Bruyn-van Ekenstein rearrangement of an α-hydroxy carbonyl group. This rearrangement inhibits reversal of the Michael addition with loss of the cyanide. It is unclear at which stage hydrolysis of the acetate occurs.

The structure of the nitrile-containing minor metabolite (**3**) was established by recourse to 2D NMR data and comparison of the IR and the ^{1}H and ^{13}C NMR data (Table 2) of this compound with those of **2**. Eight of the nine degrees of unsaturation implied by the molecular formula of

3 ($C_{21}H_{27}NO_3$) were attributed to the nitrile moiety, the furanolabdane skeleton and the carbonyl functionality (δ_C 193.6). The single remaining double bond equivalent was assigned to a tetra-substituted olefin from the chemical shifts of two vinylic quaternary carbons (δ_C 125.0 and 140.0) in the ^{13}C NMR spectrum of **3** (Table 2). Key HMBC correlations (Fig. 1) positioned the carbonyl moiety at C-6 and confirmed that this group was conjugated with a Δ^7-enolic olefin. An X-ray structural analysis of **3** (Fig. 2) unequivocally confirmed the C-9α position of the nitrile substituent proposed from a NOESY correlation between the C-11 methylene protons (δ_H 1.85 and 1.99) and the C-20 methyl protons (δ_H 1.00).

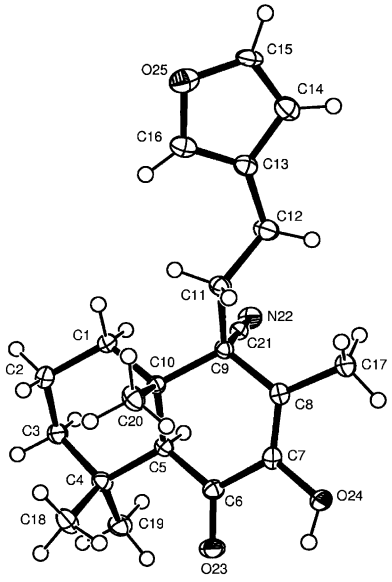


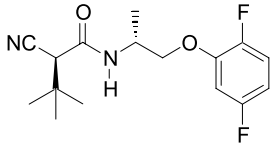
Figure 2. A view of a molecule of 9α-cyano-15,16-epoxy-7-hydroxylabda-7,13(16),14-trien-6-one (**3**) from the crystal structure showing the numbering scheme employed. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level.[19]

Preliminary in vitro screening of **2** against a panel of plant diseases suggested that this compound possessed potential anti-fungal activity. In an attempt to obtain further analogues of **2** for in planta screening against several pathogenic plant fungi, the diol nitrile **4** was prepared by LAH reduction of **2**. The β-axial assignment of the secondary alcohol moiety at C-6 followed from the small coupling constants ($J_{5,6}=1.6$ Hz and $J_{6,7}=3.1$ Hz) between the H-6 proton (δ_H 4.28) and H-5 (δ_H 1.44) and between H-6 and H-7 (δ_H 3.53).

Table 3. One day protectant percent disease control of compounds **2**, **3** and **4** against *M. grisea* and *P. recondita*

Compound	Concentration (ppm)	% Control of *M. grisea*	% Control of *P. recondita*
2	200	90	80
	50	33	22
	12.5	21	22
3	200	91	—
4	200	83	56
	50	29	22
	12.5	13	0
Tebuconazole	25	63	100
Azoxystrobin	50	99	99
	25	99	100
	12.5	99	100

Compounds **2**–**4** all exhibited significant control of rice blast (*M. grisea*) at a concentration of 200 ppm in initial in planta screens. Interestingly, **2** also exhibited an 80% control of wheat brown rust fungus *Puccinia recondita* f.sp. *tritici* at this concentration. Unfortunately, the ability of both **2** and **4** to prevent the infection of plants by *M. grisea* or *P. recondita* declined with dilution. This trend was not observed in the commercial fungicide (e.g., azoxystrobin) control experiments (Table 3). A paucity of **3** prevented the acquisition of similar dose response data for this compound.



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Compounds possessing both a nitrile functionality and activity against rice blast are not unprecedented. The nitrile (**15**) and a series of related analogues exhibited excellent control of rice blast disease in outdoor field trials.[20,21] Interestingly, the role of **15** in the control of *M. grisea* infection has been linked to this compound's ability to inhibit the enzyme scytalone dehydratase (SD) necessary for the biosynthesis of fungal melanin in rice blast.[20] The production of fungal melanin facilitates the penetration of the rice leaf by *M. grisea* and therefore, enhances infection of the plant by this pathogenic fungus. The SD inhibition of **2**, **3** and **4**, if any, is unknown.

3. Conclusion

The abundant furanolabdane diterpene metabolite hispanolone **1**, isolated from the endemic South African plant *B. africana*, continues to provide a suitable starting point for the semi-synthesis of a number of bioactive diterpene compounds. In this paper the semi-synthesis of **2** and **3**, arising from a novel tandem Michael addition/Lobry de Bruyn–van Ekenstein rearrangement of both 6α and 6β-acetoxy analogues (**9** and **10**) of dehydrohispanolone **5**, is described. Both **2** and **3** and the reduction product of **2**, diol nitrile **4**, exhibited >80% inhibition of the pathogenic plant fungus, *M. grisea* (rice blast) at a concentration of 200 ppm.

4. Experimental

Diaion HP-20 polystyrene beads (supplied by Supelco) and Kieselgel 60 (230–400 mesh) were used for initial chromatographic separations. Semi-preparative HPLC was performed using a Whatman's Partisil 10 column (10 mm i.d., length 50 cm). Optical rotations were measured using a Perkin-Elmer 141 polarimeter at the sodium *D* line (598 nm). IR spectra were recorded on a Perkin Elmer Spectrum 2000 FT-IR spectrometer with the compounds as films (neat) on NaCl discs. The NMR spectra were acquired on a Bruker AVANCE 400 MHz spectrometer using

standard pulse sequences. Chemical shifts are reported in ppm, referenced to residual solvent resonances ($CDCl_3$ δ_H 7.25, δ_C 77.0), and coupling constants are reported in Hz. HRFABMS data were obtained on a JEOL SX102 spectrometer.

4.1. Isolation of hispanolone (1) from *B. africana*

Aerial parts of *B. africana* were collected 40 kms south west of Grahamstown, South Africa in November 2003. Air-dried leaves (250 g) were steeped in Me_2CO (3.5 L) for 3 days, the Me_2CO solution concentrated in vacuo, decolourised with activated charcoal (20 g), adsorbed onto HP-20 beads and eluted sequentially with aliquots (1.5 L) of 40 and 60% aqueous acetone. The fraction eluted with 60% aqueous acetone was diluted with H_2O (1.5 L) and allowed to stand at 4 °C for 4 days to afford **1** (3.82 g, 1.52%) as white crystalline plates (acetone/water); mp 133–135 °C, lit.[3] 142–144 °C; $[\alpha]_D^{32}$ −18.8 (*c* 6.35, $CHCl_3$), lit.[3] −17.6; IR, ^1H and ^{13}C NMR data consistent with published values.[3,8]

4.2. Dehydration of 1 and α-acetoxylation of hispanone (5)

The procedures for the dehydration of **1** to give **5** and the subsequent α-acetoxylation of **5** to give the epimeric acetates **9** and **10** have been previously reported.[13]

4.3. Saponification of 9 and 10

A solution of **9** (163 mg, 0.46 mmol) in EtOH (30 mL) and 1.5 M KOH (5.0 mL) was heated at 70 °C for 1 h. The reaction mixture was cooled, 1.0 M HCl (10.0 mL) added and the EtOH removed in vacuo to give a cloudy suspension that was extracted with CH_2Cl_2 (3×15 mL). The organic phases were combined, washed with 5% aqueous $NaHCO_3$ (10 mL), dried (anhydrous $MgSO_4$) and concentrated to give a yellow oil (106 mg). Column chromatography (4:1 hexane/EtOAc) and normal phase semi-preparative HPLC of the oil (19:1 hexane/EtOAc) gave **7** (7 mg, 4%),[13] (**8**, 45 mg, 30%)[13] and **11** (38 mg, 26%). The saponification procedure was repeated on **10** (58 mg, 0.16 mmol) and the same products **7**, **8** and **11** were obtained in similar yields (6, 39 and 38%).

4.3.1. 11,12-Dihydro-7-hydroxyhedychenone (11). Yellow oily solid; $[\alpha]_D^{25}$ +2 (*c* 0.89, $CHCl_3$), lit.[16] +0.7; IR ν_{max} 3382 (br), 2930, 2863, 1692, 1615, 1615, 1471, 1385, 1026, 874, 774 cm^{-1}; ^1H and ^{13}C NMR data presented in Table 1; EIMS *m/z* (rel. int.) 316 [M^+] (6), 301 (100), 283 (43), 255 (17), 229 (12), 192 (17), 175 (12), 161 (26), 133 (14), 96 (11); HRFABMS *m/z* 317.2116 (calcd for $C_{20}H_{29}O_3$ [$(M+H)^+$], 317.2117).

4.4. KCN mediated hydrolysis of 9 and 10

A solution of a 5:2 mixture of **9** and **10** (152 mg, 0.4 mmol) and KCN (70 mg, 1.08 mmol) in 95% EtOH (3 mL) was refluxed (24 h). The ethanol was removed in vacuo, and the white residue taken up in water (5 mL) and extracted with EtOAc (3×5 mL). The organic fractions were combined, dried (anhydrous $MgSO_4$) and concentrated to give a pale yellow oil (150 mg) that after normal phase HPLC (9:1 hexane/EtOAc) gave **2** (65 mg, 45%), **3** (14 mg, 9%), **7** (2 mg, 1%)[13] and **8** (3 mg, 2%).[13]

4.4.1. 9α-Cyano-15,16-epoxy-7β-hydroxylabda-13(16), 14-dien-6-one (2). White amorphous solid; $[\alpha]_D$ +53 (*c* 0.80, $CHCl_3$), IR ν_{max} 3469, 2980, 2874, 2228, 1715, 1464, 1366, 1046, 874, 780 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; EIMS *m/z* (rel. int.) 343 [M^+] (6), 326 (17), 314 (5), 262 (8), 182 (21), 139 (29), 121 (25), 95 (100), 67 (46); HRFABMS *m/z* 344.2226 (calcd for $C_{21}H_{30}NO_3$ [$(M+H)^+$], 344.2226).

4.4.2. 9α-Cyano-15,16-epoxy-7-hydroxylabda-7,13(16), 14-trien-6-one (3). White needles (from benzene/hexane); mp 138–139 °C; $[\alpha]_D$ −44 (*c* 0.73, $CHCl_3$); IR ν_{max} 3397 (br), 2927, 2863, 2351, 2222, 1683, 1456, 1386, 1122, 876, 792 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; EIMS *m/z* (rel. int.) 341 [M^+] (13), 326 (100), 243 (12), 167 (15), 95 (17), 81 (19), 67 (15), 55 (11); HRFABMS *m/z* 341.1992 (calcd for $C_{21}H_{27}NO_3$ [$(M)^+$], 341.1990).

4.5. LAH reduction of 2

A solution of $LiAlH_4$ (3.6 mg, 0.038 mmol) and **2** (13 mg, 0.03 mmol) in dry THF (4 mL) was refluxed (5 h), cooled and acidified. The solvent was removed in vacuo and the residue taken up in EtOAc (5 mL), washed with H_2O (3× 2 mL), dried (anhydrous $MgSO_4$) and concentrated to yield a yellowish oil (17 mg). Subsequent purification with normal phase HPLC (4:1 hexane/EtOAc) yielded **4** (13 mg, 0.038 mmol, 98%).

4.5.1. 6,7-Hydroxy-9-carbonitrile-15,16-epoxylabda-13(16),14-dienol (4). Colourless oil; $[\alpha]_D^{19}$ +37 (*c* 0.5, $CHCl_3$); IR ν_{max} 3437, 2928, 2859, 2852, 2221, 1365, 1160, 1023, 871, 784 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.03 (3H, m, H_3-18 or H_3-19); 1.25 (3H, m, H_3-19 or H_3-18); 1.26 (3H, m, H_3-20); 1.27 (3H, m, H_3-17); 1.28 (1H, m, H-3a); 1.40 (1H, m, H-3b); 1.44 (1H, d, J=1.6 Hz, H-5); 1.53 (1H, dd, J=6.4, 2.7 Hz, H-11a); 1.55 (1H, m, H-2a); 1.60 (2H, m, H_2-1), 1.66 (1H, m, H-2b); 1.96 (1H, ddd, J= 14.4, 12.5, 6.9 Hz, H-11b); 2.11 (1H, m, H-8); 2.65 (2H, m, H_2-12); 3.53 (1H, dd, J=10.9, 3.5 Hz, H-7); 4.28 (1H, dd, J=3.1, 1.7 Hz, H-6); 6.27 (1H, d, J=1.8 Hz, H-14); 7.24 (1H, br s, H-16); 7.35 (1H, t, J=1.5 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.2 (q, C-17); 17.1 (q, C-20); 18.6 (t, C-2); 24.5 (q, C-18 or C-19); 24.6 (t, C-12); 31.7 (t, C-11); 33.4 (q, C-19 or C-18); 34.4 (s, C-4); 37.6 (t, C-1); 38.0 (d, C-8); 41.9 (s, C-10); 43.0 (t, C-3); 51.2 (d, C-5); 54.1 (s, C-9); 69.6 (d, C-6); 74.3 (d, C-7); 110.6 (d, C-14); 121.4 (s, C-21); 124.0 (s, C-13); 138.7 (d, C-16); 143.0 (d, C-15); HRFABMS *m/z* 345.2304 (calcd for $C_{21}H_{31}NO_3$ [$(M+H)^+$], 345.2304).

4.6. In planta test methods

For evaluations in 1 day protectant tests, samples of the compounds were dissolved in acetone at 2000 ppm, then diluted in acetone to 500 and 125 ppm. Samples were then brought to final concentrations of 200, 50 and 12.5 ppm by addition of 9 volumes of milli-Q water containing 110 ppm Triton X-100. Applications were made in 20 mL spray

volumes using a turntable sprayer equipped with two Spraying Systems Co. 1/4JAUPM-SS nozzles with 4010055 fluid caps and a spray pressure of 206 kPa.

Inoculation of plants was done by spraying suspensions of 5×10^5–6×10^6 conidia per mL, depending on the pathogen, 1 day after application. Conidial suspensions were prepared with de-ionized water containing 3 drops of Tween-20 per 100 mL of suspension. Inoculated plants were placed in a dew room (99–100% relative humidity, 20 °C) overnight to allow infection. The plants were then moved to either a greenhouse or growth chamber at a suitable temperature, daylength and humidity for expression of the disease.

Evaluation of disease control were made 6 days after inoculation in the case of *M. grisea* and 7 days after inoculation in the case of *P. recondite*, by visual estimation of disease severity. Percent disease control was calculated from disease severity ratings by the calculation: $\%DC=(1-\%DS_{trt}/\%DS_{untrt})\times100$, where %DC is percent disease control; $\%DS_{trt}$ is the observed percent disease severity for treated plants; and $\%DS_{untrt}$ is the observed percent disease severity for untreated controls.

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References and notes

1. Talbot, N. J. *Trends Microbiol.* **1995**, *3*, 9–16.
2. Ou, S. H. *Rice Diseases*, 2nd ed.; CAB: Surrey, UK, 1985; p 110.
3. Savona, G.; Piozzi, F. *Heterocycles* **1978**, *9*, 257–261.
4. Savona, G.; Bruno, M.; Piozzi, F.; Barbagallo, C. *Phytochemistry* **1982**, *21*, 2132–2133.
5. Çitoglu, G.; Tanker, M.; Sever, B.; Englert, J.; Anton, R.; Altanlar, N. *Planta Med.* **1998**, *64*, 484–485.
6. Rodríguez, B.; Savona, G. *Phytochemistry* **1980**, *19*, 1805–1807.
7. Hon, P. M.; Wang, E. S.; Lam, S. K. M.; Choy, Y. M.; Lee, C. M.; Wong, H. N. C. *Phytochemistry* **1993**, *33*, 639–641.
8. Davies-Coleman, M. T.; Rivett, D. E. A. S. *Afr. J. Chem.* **1990**, *43*, 117–119.
9. Garcia-Alvarez, M. C.; Pérez-Sirvent, L.; Rodríguez, B.; Bruno, M.; Savona, G. *An. Quim.* **1981**, *77*, 316–319.
10. Pérez-Sirvent, L.; Garcia-Alvarez, M. C.; Balestrieri, M. A.; Rodríguez, B.; Savona, G. *An. Quim.* **1981**, *77*, 330–334.
11. Dominguez, G.; Marco, J. A.; Hueso-Rodríguez, J. A.; Rodríguez, B. *An. Quim.* **1988**, *84*, 211–215.
12. Hueso-Rodríguez, J. A.; Dominguez, G.; Rodríguez, B. *An. Quim.* **1988**, *84*, 215–218.
13. Gray, C. A.; Rivett, D. E. A.; Davies-Coleman, M. T. *Phytochemistry* **2003**, *63*, 409–413.
14. Tanyeli, C.; Tosun, A.; Turkut, E.; Sezen, B. *Tetrahedron* **2002**, *59*, 1055–1058.
15. Demir, A. S.; Reis, O.; Igdir, A. C. *Tetrahedron* **2004**, *60*, 3427–3432.
16. Sharma, S. C.; Tandon, J. S.; Dhar, M. M. *Phytochemistry* **1976**, *15*, 827–828.
17. Crombie, L.; Godin, P. J. *J. Chem. Soc.* **1961**, 2861–2876.
18. Mori, K.; Tominaga, M.; Takigawa, T.; Matsui, M. *Synthesis* **1973**, 790–791.
19. X-ray analysis of **3**. Intensities were recorded from a cubic fragment using Φ- and ω-scans on a Nonius Kappa CCD diffractometer employing Mo K_α-radiation with the crystal cooled to 113(2) K in a nitrogen stream. Crystal data for **3**: $C_{21}H_{27}NO_3$, $M=341.44$, monoclinic, space group $P2_1$ (no. 4), $a=6.2718(1)$ Å, $b=14.6696(2)$ Å, $c=9.7782(2)$ Å, $\beta=103.735(1)°$, $V=873.92(3)$ Å^3, $Z=2$, $D_c=1.298$ Mg/m^3, μ(Mo K_α)$=0.086$ mm^{-1}, $F(000)=368$. A total of 3700 reflections were collected, of which 3410 were observed [$I>2\sigma(I)$]. The structure was solved by direct methods and refined on F^2 using all data, with non-hydrogen atoms treated anisotropically. All H atoms were located but were added in idealised positions in a riding model. The final R factors were $R_1=0.0385$ (all data), 0.0334 (observed data), $wR_2=0.0846$ (all data), 0.0816 (observed data) for 231 parameters. Crystallographic data (excluding structure factors) for this compound have been deposited at the Cambridge Crystallographic Data Centre as supplementary material no. 262101.
20. Jordan, D. B.; Lessen, T. A.; Wawrzak, Z.; Bisaha, J. J.; Gehert, T. C.; Hansen, S. L.; Schwartz, R. S.; Basarab, G. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1607–1612.
21. Basarab, G. S.; Jordan, D. B.; Gehert, T. C.; Schwartz, R. S.; Wawrzak, Z. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1613–1618.