Synthesis of NH006—a photostable fungicide effective against *Botrytis cinerea*—according to the asymmetric total synthesis of MK8383†

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MK8383, isolated from *Phoma* sp. T2526 in 1993, exhibits potent antibiotic activities against a variety of phytopathogens and has been considered a promising fungicide against *Botrytis cinerea*. Unfortunately, MK8383 is a photosensitive compound and it undergoes irreversible decomposition. Although much effort has been devoted to improving the photostability of MK8383 by chemical modification of its structure by a research group organized by Meiji Seika Kaishya, Ltd. and Mitsubishi Chemical Corporation, a photostable MK8383 derivative has never been prepared. We have found that a C13-14 double bond of MK8383 and (+)-phomopsidin is responsible for the photosensitivity, and herein, we report the synthesis of NH006, an MK8383 derivative with a saturated C13-14 double bond and (*S*) configuration at C14, based on the asymmetric total synthesis of MK8383. NH006 exhibits good photostability and potent antifungal activity against *B. cinerea.*

Introduction

In 1993, a research group organized by Meiji Seika Kaishya, Ltd. and Mitsubishi Chemical Corporation isolated MK8383 (Fig. 1) from *Phoma* sp. T2526 as a new fungicide.[1] This compound has a *cis*-fused dehydrodecalin ring with two characteristic substituents, (*E*,*E*)-pentadienoic acid and a (*Z*)-trisubstituted alkene. The structure of MK8383 is almost the same as that of (+)-phomopsidin[2] (Fig. 1), except for the geometry of the trisubstituted side-chain alkene.

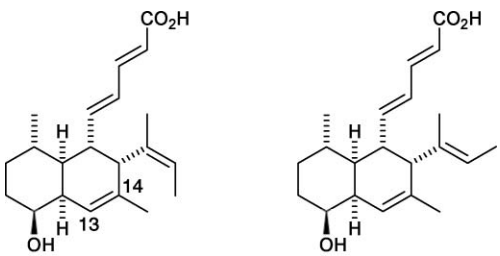

Fig. 1 Structures of MK8383 and (+)-phomopsidin.

In comparison with (+)-phomopsidin (IC_{50} of 5.7 μM),[2] MK8383 shows relatively weak inhibitory activity (IC_{50} of 8.0 μM) against an assembly of microtubule proteins derived from porcine brains. However, the antibiotic activity of MK8383 against a variety of phytopathogens, especially against *Botrytis cinerea*, is more potent than that of (+)-phomopsidin. The activity of MK8383 is comparable to that of the commercially available fungicides.[1]

B. cinerea is a facultative phytopatogenic fungus that attacks flowers, fruits, leaves, and stems of more than 200 plant species, causing grey mould disease.[3] Dicarboximide, benzimidazole, and several fungicides are known to be effective against *B. cinerea*; however, the continuous use of such commercially available fungicides has resulted in the development of highly resistant strains of *B. cinerea* as well as the contamination of soil and water.[4] Some natural products have been reported to show antifungal activity against *B. cinerea*; therefore, such compounds are expected to be used as alternative fungicides to the commercial ones.[5]

MK8383 and its derivatives are considered promising fungicides against *B. cinerea*;[1] however, MK8383 is a light-sensitive compound and it undergoes irreversible decomposition (Fig. 2, Table 1). Although much effort has been devoted to finding photostable derivatives of MK8383 that show high antifungal activity against *B. cinerea*, no MK8383 derivatives with photostability have been prepared. Thus, the development of a new fungicide derived from MK8383 is desired.

Table 1 Residual ratio (%) after irradiation of artificial sunlight lamp[a]

Time (h)	MK8383	(+)-phomopsidin	**12**[b]	**16**[b]	NH006[b]
0	100.0	100.0	100.0	100.0	100.0
1	99.7	88.9	98.9	103.2	99.4
2	99.0	89.9	106.2	102.1	99.6
4	94.4	77.4	103.4	102.9	100.4
8	78.7	59.8	100.0	101.5	101.8
16	51.4	39.2	99.6	101.1	96.2
24	30.1	19.5	94.2	103.0	87.5
Control	107.0	100.0	100.2	99.8	106.0

[a] See experimental. [b] Average value of three tests.

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† Electronic supplementary information (ESI) available: Data of new compounds and experimental procedures. CCDC reference number 751813. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b924096a

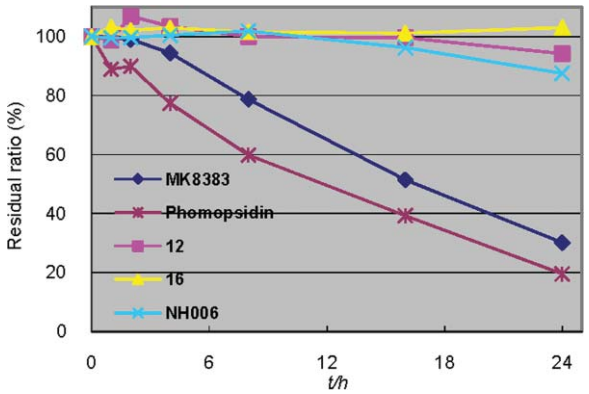


Fig. 2 Results of the photostability test of MK8383, (+)-phomopsidin, **12**, **16**, and NH006.

In 2003, we reported on the first total synthesis of (+)-phomopsidin based on the construction of a *cis*-fused dehydrodecaline skeleton by a stereoselective transannular Diels–Alder (TADA) reaction.[6] Recently, we also achieved the asymmetric total synthesis of MK8383 *via* the iron-mediated stereoselective construction of a (*Z*)-trisubstituted alkenyl side chain.[7a] As the number of derivatives obtained from a natural product is limited, we focused on the preparation of MK8383 derivatives with photostability and high antifungal activity based on the total syntheses of (+)-phomopsidin and MK8383. Recently, we succeeded in the preparation of NH006, a promising fungicide against *B. cinerea* with photostability. In this paper, we discuss its preparation and its properties such as photostability and antifungal activity.

We surmised that the photosensitivity of MK8383 could be attributed to the double bonds existing in the dehydrodecalin core and side chains. However, previous studies on the relationship between the structure of MK8383 and its photostability have suggested that the photosensitivity of MK8383 cannot be attributed to the double bonds in the side chains.[1] Thus, the C13-C14 double bond in the dehydrodecalin core has been suspected to be a primary cause for the photosensitivity of MK8383.

The selective modification of a C13-C14 double bond was difficult in the presence of side chain double bonds; therefore, an MK8383 derivative with a modified C13-C14 double bond has never been prepared.[1] Hence, we decided to modify the C13-C14 double bond on the basis of the asymmetric total syntheses of (+)-phomopsidin and MK8383.

(+)-Phomopsidin exhibits relatively weak antifungal activity against *B. cinerea* in comparison with MK8383, but it can be synthesized more easily than MK8383 (Fig. 4, Table 2). Hence, we prepared (+)-phomopsidin derivatives with a modified C13-C14 double bond and then studied the effect of structural modification on the photostability and antifungal activity of the derivative in order to develop a method for preparing an MK8383 derivative.

Results and discussion

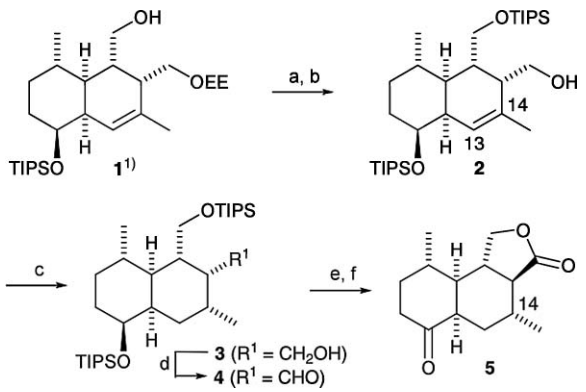
Synthesis, antifungal activity, and photo-stability of compound 12

A (+)-phomopsidin derivative with a saturated C13-C14 bond was expected to show the desired photostability. Therefore, we carried out the hydrogenation of compound **1**, which was a synthetic intermediate in our asymmetric total syntheses of (+)-phomopsidin and MK8383. However, hydrogenation of **1** gave a mixture of diastereomers under all reaction conditions. Therefore, **1** was converted to the corresponding triisopropyl (TIPS) ether. This was followed by removal of the ethoxyethyl group to give alcohol **2**, which was subjected to hydrogenation in the presence of Rh/Al_2O_3 catalyst to successfully afford compound **3** as a single product (84%, 86% conv.) (Scheme 1). Dess–Martin oxidation[8] of compound **3**, subsequent treatment of the resulting aldehyde **4** with tetrabutylammonium fluoride (TBAF) to remove the TIPS group, and Dess–Martin oxidation for a second time afforded lactone **5**.

Table 2 Protective value (%) against *Botrytis cinerea* evaluated by cabbage disk test[a]

Conc (ppm)	MK8383	(+)-Phomopsidin	**12**	**16**	NH006
100	90.9	100.0	8.3	83.3	100.0
25	100.0	72.3	16.7	50.0	91.7
6.25	90.9	63.1	0.0	25.0	75.0
1.56	63.6	16.9	8.3	16.7	25.0
0.39	36.4	16.9	0.0	8.3	0.0
0	0.0	0.0	0.0	0.0	0.0

[a] See experimental. Protective value was calculated using the average value of four tests.



Scheme 1 *Reagents and conditions*: a) TIPSOTf, DBU, CH_2Cl_2, –78 °C, 96%; b) AcOH/EtOH (1/5, v/v), 60 °C, 75%; c) H_2 (1 atm), $Rh\text{-}Al_2O_3$, MeOH/AcOH (5/1, v/v), rt, 84% (86% conv.); d) Dess–Martin periodinane, CH_2Cl_2, rt, 95%; e) TBAF, THF, reflux; f) Dess–Martin periodinane, CH_2Cl_2, rt, 40% (2 steps).

Lactone **5** was crystalline, and the single crystal formed was suitable for the X-ray crystallographic analysis. The X-ray structure[9] shown in Fig. 3 indicates that epimerization occurred during the transformation of aldehyde **4** to lactone **5**; moreover, the configuration of C14 was unambiguously confirmed. This X-ray crystallographic analysis suggested that the hydrogenation of **2** with Rh/Al_2O_3 catalyst occurred opposite to the C15 hydroxymethyl group. Therefore, this stereoselectivity would be well explained by the reaction occurring at the less hindered side rather than the reaction directed by the adjacent C15 hydroxymethyl group.

Aldehyde **4** was subjected to the Corey–Fuchs protocol[10] to afford alkyne **6**. Carboalumination of **6** under Wipf's conditions[11]

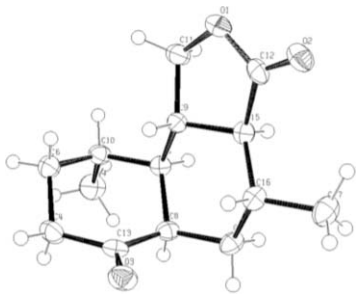


Fig. 3 X-Ray structure of **5**. Displacement ellipsoids are drawn at the 50% probability level.

and in situ trapping of the product with iodine gave iodide **7**. Negishi coupling of iodide **7** with dimethylzinc[12] and the subsequent treatment of the product with TBAF afforded diol **8**, followed by selective TBS ether formation and benzoylation to give compound **9**. The treatment of **9** with TBAF and subsequent Dess–Martin oxidation afforded aldehyde **10**. Then, **10** was subjected to the Horner–Wadsworth–Emmons reaction with phosphonate **11**. Hydrolysis of the resultant ethyl ester with concomitant removal of the benzoyl group afforded compound **12**.

Expectedly, **12** remained almost intact after irradiation by artificial light for 24 h (Fig. 2, Table 1), but it exhibited almost no antifungal activity even at a concentration of 100 ppm (Fig. 4, Table 2). Thus, hydrogenation of the C13-14 double bond was found to greatly improve the photostability of the (+)-phomopsidin derivative. Molecular modeling of **12** showed that its three-dimensional structure is considerably different from that of (+)-phomopsidin, but the C14 epimer of **12** has a three-dimensional structure that is similar to that of (+)-phomopsidin. As a result, we focused on the total synthesis of the C14 epimer of **12**.

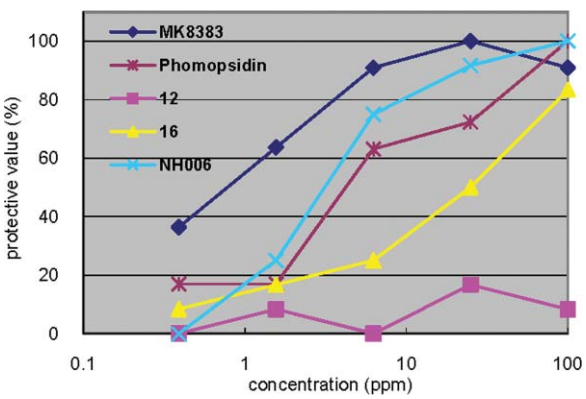
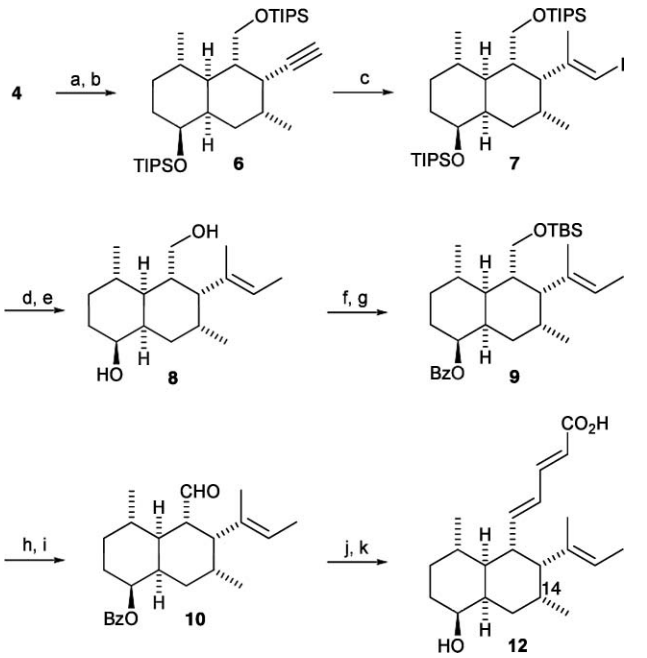
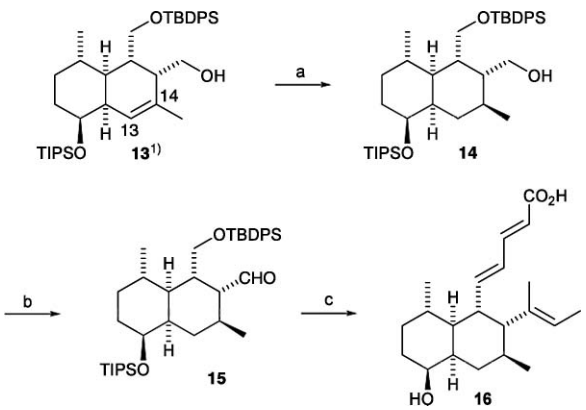


Fig. 4 Results of the antifungal activity test of MK8383, (+)-phomopsidin, **12**, **16**, and **NH006**.

To synthesize the C14 epimer of **12**, we had to establish a method for preparing compound **14** (Scheme 3) on the basis of the stereoselective hydrogenation of **13**, which was also a synthetic intermediate in the asymmetric total syntheses of (+)-phomopsidin and MK8383. We expected that the hydroxymethyl group in **13** would direct the hydrogenation to afford the desired product with high stereoselectivity. Indeed, stereoselective hydrogenation was successfully realized by the use of Crabtree's catalyst[13] to



Scheme 2 *Reagents and conditions*: a) CBr_4, PPh_3, CH_2Cl_2, rt, 84%; b) *n*-BuLi, THF, −78 °C, 88%; c) Cp_2ZrCl_2, Me_3Al, CH_2Cl_2, H_2O, −30 °C, then, I_2, THF, −30 °C, 83%; d) Me_2Zn, $PdCl_2(PPh_3)_2$, THF, rt, 96%; e) TBAF, THF, reflux, 100%; f) TBSCl, imidazole, CH_2Cl_2, rt, 71%; g) Bz_2O, DMAP, CH_2Cl_2, rt, 100%; h) TBAF, THF, rt, 98%; i) Dess–Martin periodinane, CH_2Cl_2, rt, 90%; j) $(EtO)_2P(O)CH_2CH{=}CHCO_2Et$ (**11**), LHMDS, THF, −78 to 0 °C, 91%; k) $LiOH{\cdot}H_2O$, $EtOH/H_2O$ (4/1, v/v), 89%.



Scheme 3 *Reagents and conditions*: a) H_2 (1 atm), $[Ir(cod)pyr(PCy_3)]PF_6$ (20 mol%), $(CH_2Cl)_2$ (degassed), 60 °C, 84%; b) Dess–Martin periodinane, CH_2Cl_2, rt, 95%; c) see ESI.

give **14** as the sole product. Although **14** and **3** have different protective groups on hydroxyls, the spectroscopic data of **14** was very different from that of **3**, suggesting that the C14 configuration of **14** was different from that of **3**. Therefore, we continued the synthesis of the (+)-phomopsidin derivative from **14**.

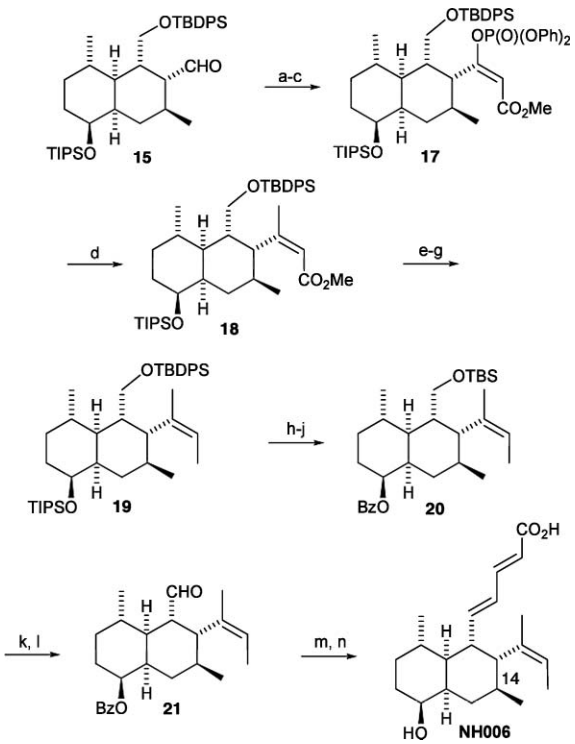
Synthesis, antifungal activity, and photo-stability of compound 16

Dess–Martin oxidation of **14** afforded aldehyde **15** that was successfully converted to compound **16** according to the method used for the preparation of **12**, which is shown in Scheme 2.

16 also showed excellent photostability (Fig. 2, Table 1); its antifungal activity was stronger than that of **12** (Fig. 4, Table 2), but weaker than that of (+)-phomopsidin.

Synthesis, antifungal activity, and photo-stability of NH006

The above results prompted us to further pursue the synthesis, photostability test, and antifungal activity test of a derivative of MK8383 because the antifungal activity of MK8383 is stronger than that of (+)-phomopsidin, and the sole structural difference between MK8383 and (+)-phomopsidin is the geometry of the trisubstituted side-chain alkene. Therefore, we initiated the synthesis of NH006, a derivative of MK8383 with a saturated C13-14 bond and whose C14 configuration is the same as that of **16**.



Scheme 4 *Reagents and conditions*: a) LDA, AcOMe, THF, −78 °C; b) Dess–Martin periodinane, CH_2Cl_2, rt, 84% (2 steps); c) Et_3N, HMPA, 0 °C, then $ClP(O)(OPh)_2$, DMAP, rt, 98%; d) MeMgCl, $Fe(acac)_3$, NMP, 0 °C, 91%; e) DIBAL, CH_2Cl_2, −78 °C, 98%; f) PBr_3, pyridine, Et_2O, rt,; g) $LiAlH_4$, Et_2O, rt, 73% (2 steps); h) TBAF, THF, reflux, 92%; i) TBSCl, imidazole, CH_2Cl_2, rt, 97%; j) Bz_2O, DMAP, CH_2Cl_2, rt, 90%; k) TBAF, THF, rt, 100%; l) Dess–Martin periodinane, CH_2Cl_2, rt, 88%; m) **11**, LHMDS, THF, −78 to 0 °C, 98%; n) $LiOH{\cdot}H_2O$, $EtOH/H_2O$ (10/1, v/v), 82%.

The total synthesis of NH006 was carried out starting from aldehyde **15** on the basis of the asymmetric total synthesis of MK8383.[7] Thus, an aldol reaction of methyl acetate with **15** and subsequent Dess–Martin oxidation of the product afforded a β–keto ester, which was converted to enol phosphate **17**. A coupling reaction of **17** with methylmagnesium bromide in a mixed solvent composed of THF and NMP in the presence of $Fe(acac)_3$ afforded compound **18** in 91% yield.[14] Reduction of **18** with DIBAL and subsequent treatment of the resultant alcohol with CBr_4 and PPh_3 resulted in the isomerization of the trisubstituted side-chain alkene; however, the use of PBr_3 in the presence of pyridine successfully suppressed the isomerization and converted the allylic alcohol to the desired bromide. The reaction of the allylic bromide with $LiAlH_4$ gave compound **19**, and subsequent treatment of **19** with TBAF afforded a diol. The primary and secondary hydroxyls of the diol were protected as TBS ether and benzoate, respectively, to give compound **20**. The TBS group of **20** was removed with TBAF, and subsequent Dess–Martin oxidation afforded aldehyde **21**. The Horner–Wadsworth–Emmons reaction of **21** with phosphonate **11** and hydrolysis of the resultant ethyl ester with concomitant removal of the benzoyl group furnished NH006.

Although NH006 slightly decomposed after 24 h irradiation (*ca.* 12.5%), NH006 showed excellent photostability, which was expected from the results of compounds **12** and **16** (Fig. 2, Table 1). Comparing the relationships between the structure and photostablity of **12**, **16**, and NH006, the slight decomposition of NH006 after 24 h irradiation could be attributed to the relatively photosensitive (*Z*)-trisubstituted side-chain alkene in NH006. The antifungal activity of NH006 was also slightly reduced as compared to that of MK8383 (Fig. 4, Table 2), but it was better than that of (+)-phomopsidin. Thus, NH006 is sufficiently potent as a fungicide against *B. cinerea*.

Conclusions

We successfully prepared NH006 that showed good photostability and potent antifungal activity against *B. cinerea*. The stereoselective hydrogenation of the C13-14 double bond of the intermediates in the total synthesis of (+)-phomopsidin and MK8383 was successfully achieved. Hydrogenation was carried out by the reaction occurring at the less hindered side using Rh/Al_2O_3 catalyst or by the reaction directed by the hydroxyl group using Crabtree's catalyst to give both diastereomers as the sole product in the respective reaction. Starting from the products thus prepared, two C13-14-saturated (+)-phomopsidin derivatives were synthesized, and the C13-14 double bond was found to be essentially responsible for the photosensitivity of (+)-phomopsidin and MK8383. The derivatives with a saturated C13-14 bond prepared in this study showed high antifungal activity when the C14 configuration was (*S*). The (*Z*) configuration of the trisubstituted side-chain alkene of NH006 was also found to play an important role in the high antifungal activity. As NH006 is a promising fungicide with good photostability, a field test of the antifungal activity of NH006 against *B. cinerea* is now underway, and the results will be reported in due course.

Experimental

Photostability test

500 μL of 200 μg/mL test compound in acetone was dispensed in glass dishes (50 mm in diameter) and dried in the dark. The dishes were exposed to artificial sunlight lump for 24 h in a chamber (25 °C, 60% RH). The illumination and ultraviolet intensity was 30,000 lux and 300–400 $\mu W/cm^2$, respectively. The dried compound was washed with 500 μL of methanol. The methanol solution was analyzed by HPLC on an XTerra C18 column

Disease index	Lesioned area on leaf disk
0	No lesioned area was recognized.
1	Lesioned area was very small.
2	Lesioned area was formed less than control.
3	Lesioned area was formed as same as control.

(dimensions: 4.5 × 50 mm) using 15%–100% CH_3CN in H_2O over 10 min (flow rate: 0.8 mL/min) to evaluate the residual ratio. The residual ratio was calculated by using following equation. rediual ratio (%) = (area exposed for 24 h)/(area not exposed)

Cabbage leaf disk test

A cabbage leaf disk test was carried out by using four disks cut from cabbage leafs (cv. Kinkei 201 EX) with a cork borer (14 mm in diameter); the leaves were grown in a greenhouse for 1 month. The leaf disks were placed in a 24-well plate (Cellstar®, No. 662160, Greiner Bio-One GmbH). The test compound solution containing 10% acetone and 0.05% v/v spreader (Neoesterin, Kumiai Chemical Industry Co., Ltd.) was sprayed on the four cabbage leaf disks (8 μL/disk), and the disks were allowed to dry naturally. A conidia suspension (1×10^5 conidia/mL) of *B. cinerea* containing 10% w/v potato tuber extract, 2.5% w/v dextrose, and 0.125% w/v inosine was inoculated into the leaf disks using a spray gun (2 mL per 24-well plate). After incubation for 3 days at 21 °C in a humid and dark chamber, the disks were assessed visually for lesioned areas and the disease index was obtained from the following table below.

Protective value: The protective value was calculated by using the following equation. The disease index is the mean of the disease indices of the four leaf disks. Protective value = ((disease index of control)–(disease index of test compound))/(disease index of control) × 100.

Acknowledgements

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9 **Crystallographic data for 5**. $C_{14}H_{20}O_3$, M = 236.31, orthorhombic, a = 7.4309(10), b = 10.1138(12), c = 16.7778(18)Å, V = 1260.9(3)Å^3, T = 123.1 K, $P21$ (#19), Z = 4, Dx = 1.245 g cm^{-3}, Mo-Ka radiation, 12303 measured reflections, 2884 independent reflections (R_{int} = 0.038), $R1$ = 0.0488 for 2254 data with $I > 2s(I)$, w$R2$ = 0.1278 (all data). Fig. 2 shows the relative stereochemistry of **5**. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data centre as supplementary publication numbers CCDC 751813. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. The atom numbers in Fig. 2 do not correspond to those in Fig. 1 and Schemes 1–4.
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