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Ti-catalyzed transannular cyclization of epoxygermacrolides. Synthesis of antifungal (+)-tuberiferine and (+)-dehydrobrachylaenolide

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ABSTRACT

We present a divergent strategy for the stereoselective synthesis of both eudesmanolides (+)-tuberiferine and (+)-brachylaenolide starting from the accessible germacrolide (+)-costunolide. The key steps of these syntheses are the Ti-catalyzed transannular cyclization of a 1,4-epoxygermacrolide in the presence or absence of water, respectively. The catalytic cycle operating in the presence of water probably involves the reduction of a tertiary radical by H-atom transfer from aquacomplex $Cp_2Ti^{III}(OH_2)Cl$. The catalytic cycle under anhydrous conditions presumably occurs through mixed disproportionation between a tertiary radical and $Cp_2Ti^{III}Cl$. Synthetic (+)-tuberiferine and (+)-brachylaenolide displayed an antifungal potency against *Phycomyces blakesleeanus* comparable or even higher than amphotericin B, the gold standard for antifungal therapy.

1. Introduction

Since the pioneering work by RajanBabu and Nugent on Ti^{III}-promoted homolytic epoxide opening,[1] biscyclopentadienyl titanium chloride[2] (Cp_2TiCl) has become a formidable tool in organic synthesis,[3] especially after the catalytic version developed by Gansäuer et al., who used 2,4,6-collidine hydrochloride as titanocene-regenerating agent.[4] Subsequently we developed an alternative, non-protic titanocene-regenerating agent, the couple Me_3SiCl/2,4,6-collidine, which in situ presumably forms *N*-(trimethylsilyl)-collidinium chloride, the actual titanocene-regenerating reagent.[5] Ti-catalyzed homolytic epoxide opening has proven to be one of the most convenient procedures to initiate radical cascade cyclizations, which have been intensively exploited for the straightforward synthesis of terpenoids, including monoterpenoids such as karahanaenone,[6] sesquiterpenoids such as *trans*-4(11),8-daucadiene,[6] isodrimenediol,[7] 3β-hydroxydihydroconfertifolin[7] and (−)-α-ambrinol,[8] diterpenoids such as 3β-hydroxymanool,[9] rostratone,[10] barekoxide,[6] laukarlaool[6] and sclareol oxide,[11] meroterpenoids such as zonarone and zonarol,[12] and triterpenoids such as 3β-hydroxymalabarica-14(26),17*E*,21-triene[9] and achilleol A.[13] In contrast, Ti-catalyzed epoxide opening has been scarcely applied to achieve radical transannular cyclizations.[5,14]

The transannular cyclization of medium-sized rings contributes to the enhancement of molecular rigidity and structural complexity, two properties often associated with biological activity in small molecules.[15] Therefore, we decided to explore the transannular cyclization of the 10-membered ring of germacrolides, sesquiterpene lactones with a germacrane skeleton.[16] We first assayed the cationic cyclization of germacrolides, 1,10-epoxygermacrolides and 4,5-epoxygermacrolides initiated by Brønsted and Lewis acids.[14a,17] Thus, we obtained mixtures of isomeric eudesmanolides[14a,17b] (sesquiterpene lactones with an eudesmane skeleton),[16] oxidized eudesmanolides[17a] or ring-contraction products,[17c] depending on the nature of the substrate and the acid employed. We subsequently began the study of radical cyclizations of 1,10-epoxygermacrolides catalyzed by Cp_2TiCl.[5,14a] Thus, we unexpectedly found that water exerted a dramatic effect on the termination step of the radical cyclization process. In fact, whereas under anhydrous conditions an eudesmanolide with an exocyclic double bond was selectively obtained, in the presence of water the corresponding reduction product was only formed.[5,14a] As at that time the reduction of a free radical by hydrogen-atom transfer from water seemed counterintuitive, the water effect observed was explained via trapping of a tertiary radical by a Cp_2TiCl species and subsequent hydrolysis of the organometallic alkyl–Ti^{IV} intermediate formed.[5] Nevertheless, we have recently found that, possibly due to steric factors, tertiary radicals are not trapped by Cp_2TiCl under the conditions employed.[14b] Moreover, we have provided theoretical and experimental evidence supporting the idea that free radicals can be effectively reduced by hydrogen-atom transfer from the aquacomplex $Cp_2Ti(OH_2)Cl$.[14b] Therefore, the catalytic cycle originally postulated[5] should be modified as depicted in Scheme 1.

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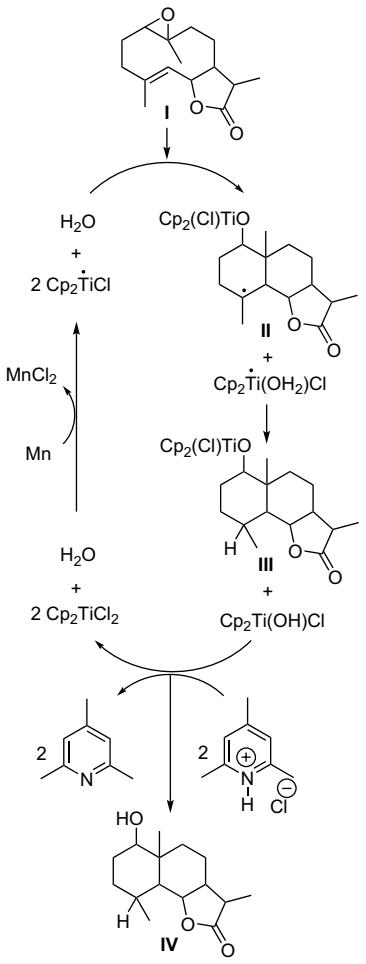


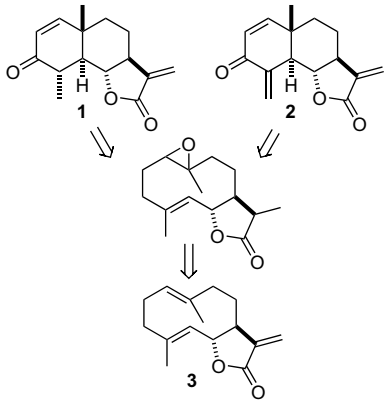
Scheme 1. Revised catalytic cycle for Ti^{III}-catalyzed transannular cyclization of 1,10-epoxygermacrolides in the presence of water.

Natural product synthesis constitutes one of the most demanding tests to prove the utility of novel synthetic methods. Therefore, once we were confident about the possibility of controlling the termination step of Ti-catalyzed transannular cyclizations of epoxygermacrolides, by means of the water effect observed, we decided taking advantage of this phenomenon for the straightforward synthesis of potentially antifungal (vide infra) eudesmanolides scarce in nature. As target molecules we chose (+)-tuberiferine (**1**), a metabolite from the plant *Sonchus tuberifer*,[18] and (+)-dehydrobrachylaenolide (**2**), isolated from the roots of *Brachylaena transvaalensis*.[19]

2. Results and discussion

To the best of our knowledge, synthetic procedures previously reported for the preparation of tuberiferin (**1**)[20] and dehydrobrachylaenolide (**2**)[21] are restricted to chemical transformations of other eudesmanolides. In contrast, our retrosynthetic analysis for both products (Scheme 2) converged to the germacrolide (+)-costunolide (**3**), which can be obtained in (multi)gram quantities from the commercially available extract Costus Resinoid.[22]

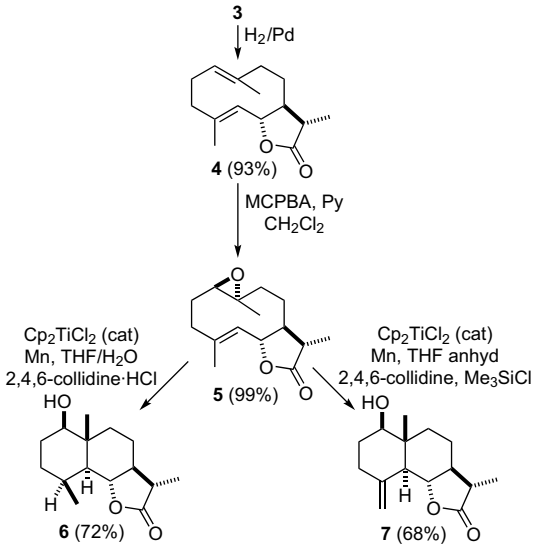
Therefore, the synthesis began with the selective reduction of the conjugated double bond of **3** to avoid potential complications with a reactive Michael-acceptor system. This double bond can easily be restored at the end of the synthetic sequence. Thus, catalytic hydrogenation of **3** by our previously described procedure[22] took place regio- and stereoselectively, giving a 93% yield of (+)-11β,13-dihydrocostunolide[23] (**4**) (Scheme 3).



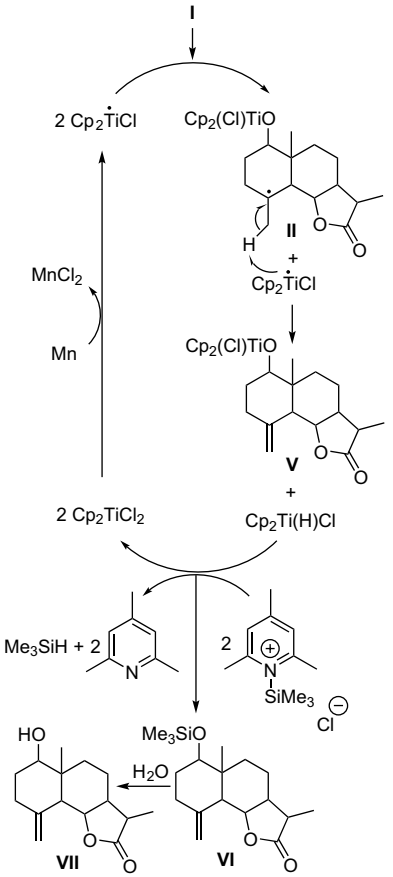
Scheme 2. Convergent retrosynthetic analysis from **1** and **2** to **3**.

Selective oxidation of **4** with *m*-chloroperbenzoic acid (MCPBA) in the presence of pyridine provided an excellent yield (99%) of epoxide **5**.[14a] At this point we took advantage of the different behaviour of the Ti(III)-catalyzed cyclization of epoxygermacrolide **5** in the presence or absence of water. Thus, the key intermediate **6** was prepared in 72% yield by stirring epoxide **5** with a substoichiometric amount of Cp_2TiCl_2 (0.2 mmol), Mn dust (7 mmol), H_2O (5 mmol), 2,4,6-collidine (5 mmol) and 2,4,6-collidine hydrochloride (5 mmol) in THF. On the other hand, treatment of **5** with Cp_2TiCl_2 (0.2 mmol), Mn (7 mmol), 2,4,6-collidine (7 mmol) and trimethylsilyl chloride (4 mmol) in anhyd THF gave exocyclic alkene **7** (68% yield). Its physical properties, including optical rotation and NMR data, matched those described for natural (+)-11β,13-dihydroreynosin isolated from the plant *Michelia compressa*,[24] supporting the usefulness of our procedure for the stereoselective synthesis of natural eudesmanolides.

It is obvious that transannular cyclization of **5** to **7**, catalyzed by Cp_2TiCl under anhydrous conditions, cannot be explained by the water-based catalytic cycle depicted in Scheme 1. We have recently demonstrated that, under anhydrous conditions, tertiary radicals are transformed into alkenes by a mixed disproportionation process promoted by Cp_2TiCl.[14b] In this scenario, anhydrous Ti-catalyzed cyclization of 1,10-epoxygermacrolides (such as model compound **I**) to eudesmanolides with an exocyclic double bond (such as model **VII**) can be rationalized by the catalytic cycle depicted in Scheme 4.



Scheme 3. Ti^{III}-catalyzed synthesis of intermediates **6** and **7**.

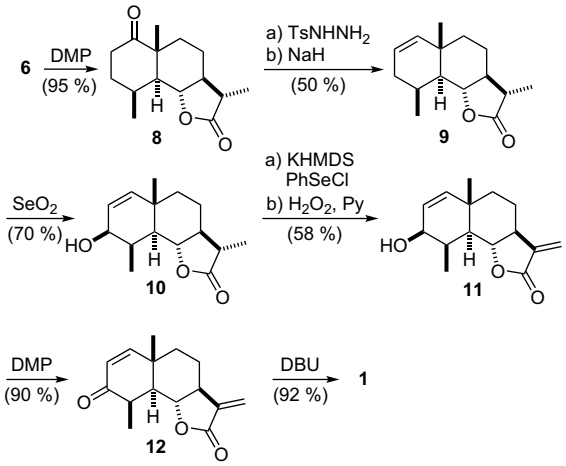


Scheme 4. Hypothetical catalytic cycle for Ti^{III}-catalyzed transannular cyclization of 1,10-epoxygermacrolides under anhydrous conditions.

Moreover, the high regioselectivity observed for the formation of exocyclic alkene **7** suggest that hydrogen-atom abstraction by the bulky Cp_2TiCl species from the methyl group of a tertiary radical such as **II** is much faster than from the methylene or the methyne groups located at the α-positions of the carbon-centred free radical. At the moment, we do not know what are the factors responsible for these different reaction rates.

Intermediate **6** was subsequently transformed into (+)-tuberiferine (**1**) in a sequence of seven steps (Scheme 5). Oxidation of secondary alcohol **6** with Dess–Martin periodinane (DMP) yielded the corresponding ketone **8** (95%). At this point, we chose the Shapiro elimination of the tosylhydrazone derived from **8** to form the $\Delta^{1,2}$ double bond characteristic of tuberiferine. In this way, we obtained the desired alkene **9** in 50% yield (two steps). Subsequent allylic oxidation of **9** provided a 70% yield of pseudo-equatorial alcohol **10**. The C-11–C-13 double bond was then restored in a one-pot reaction applying a slight modification of the selenium-based, two-step protocol described by Grieco and Nishizawa[23] Thus, we obtained a 58% yield of conjugated lactone **11**.[25] Oxidation of alcohol **11** with DMP provided conjugated ketone **12** (90% yield). Finally, base-catalyzed epimerization of the methyl group at C-4 led to the target molecule (+)-tuberiferine (**1**) (92%). Physical and spectroscopic data, including optical rotation, of synthetic **1** were in agreement with those described for the natural product.[18] Thus, we completed the synthesis of **1** from **3** in 10 steps and an 11% overall yield.

The synthesis of (+)-dehydrobrachylaenolide (**2**), from the key intermediate **7** (Scheme 6), was performed using a synthetic sequence closely related to that employed for the synthesis of **1**. Thus, DMP-based oxidation of **7** gave an 89% yield of ketone **13**. Interestingly, the Shapiro reaction of **13** gave the expected diene **14**



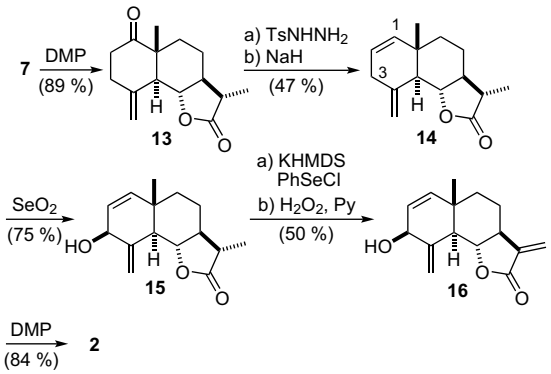
Scheme 5. Synthesis of (+)-tuberiferine (**1**).

(47%) without apparent isomerization of the exocyclic double bond. Allylic oxidation of the doubly activated C-3 position of **14** yielded allylic alcohol **15** (75%). Subsequently, the modified Grieco's protocol provided a moderate 50% yield of conjugated lactone **16**. This lactone is the C-3 epimer of (+)-brachylaenolide isolated from *B. transvaalensis*.[19] In natural brachylaenolide H-3 resonates at 4.72 ppm whereas H-3 of **16** appears at 4.43 ppm. Finally, oxidation of alcohol **16** with DMP yielded the second target molecule, (+)-dehydrobrachylaenolide (**2**). Physical and spectroscopic data of synthetic **2**, including optical rotation, were in agreement with those described for the natural product.[19] Thus, we completed the synthesis of **2** from **3** in nine steps and an 8% overall yield.

Divergent stereoselective synthesis of both eudesmanolides (+)-tuberiferine (**1**) and (+)-dehydrobrachylaenolide (**2**) from germacrolide **3** highlights the synthetic utility of Ti-catalyzed radical transannular cyclizations of 1,10-epoxygermacrolides.

3. Antifungal activity of (+)-tuberiferine (1) and (+)-dehydrobrachylaenolide (2)

In the last decades, a considerable increase of fungal infections has been observed worldwide, with special incidence of opportunistic infections in inmunodeficient patients and individuals infected by multidrug-resistant fungal strains.[26] Therefore, the development of novel, more effective antifungal drugs is desirable.[26] In this context, Oltra et al. observed that sesquiterpene lactones require a relatively low polarity and, at least, one Michael-acceptor system to display antifungal activity.[27] As both sesquiterpene lactones **1** and **2** fitted these requirements, we decided to check their



Scheme 6. Synthesis of (+)-dehydrobrachylaenolide (**2**).

potential antifungal properties. For this purpose, we chose *Phycomyces blakesleeanus*, a filamentous fungus the metabolites of which were previously analyzed in our laboratory.[28] *Phycomyces* was incubated in minimal medium[29] (control experiment) and in minimal media supplemented with different concentrations of (+)-tuberiferine (**1**), (+)-brachylaenolide (**2**) and a reference antifungal drug, amphotericin B, which is considered the 'gold standard' for antifungal therapy.[26] At the end of the incubation period (7 days), mycelia were filtered off from culture broths, lyophilized and weighted. Comparison of the biomass weights of mycelia from cultures containing antifungal products with that of the control experiment allowed the determination of the concentration required to achieve a 50% of fungal growth inhibition (GI_{50}). Thus, we determined the following values: GI_{50}=12 μg/mL for amphotericin B, GI_{50}=12 μg/mL for (+)-tuberiferine and GI_{50}=6 μg/mL for (+)-brachylaenolide. Despite of a more in-depth antifungal analysis is obviously needed; the above values indicate that, at least for *P. blakesleeanus*, lactones **1** and **2** have an antifungal potency comparable or even higher than amphotericin B.

4. Conclusions

We have developed a divergent procedure for the stereoselective synthesis of the eudesmanolides (+)-tuberiferine (**1**) and (+)-brachylaenolide (**2**), which are scarce in nature, starting from the accessible germacrolide (+)-costunolide (**3**). The key steps of these syntheses were the Ti-catalyzed transannular cyclizations of 1,4-epoxygermacrolide **5** in the presence or absence of water, respectively. The catalytic cycle operating in the presence of water probably involves the reduction of a tertiary radical by H-atom transfer from aquacomplex $Cp_2Ti^{III}(OH_2)Cl$. The catalytic cycle under anhydrous conditions presumably occurs through mixed disproportionation between a tertiary radical and $Cp_2Ti^{III}Cl$. Synthetic (+)-tuberiferine (**1**) and (+)-brachylaenolide (**2**) displayed an antifungal potency against *P. blakesleeanus* comparable or even higher than amphotericin B.

At the moment, we are engaged in the study of Ti-catalyzed transannular cyclizations of 4,5-epoxygermacrolides and in a more in-depth study of the antifungal activity of lactones **1** and **2**.

5. Experimental section

5.1. General details

For all reactions employing titanocene, solvents and additives were thoroughly deoxygenated prior to use. The numbering used corresponds to the germacrane and eudesmane sesquiterpene systems and not to the IUPAC nomenclature.[16] (+)-Costunolide (**3**) can be obtained in (multi)gram quantities from the commercially available extract Costus Resinoid (Pierre Chauvet S.A., Seillans, France) as described elsewhere.[17b] We followed previously described procedures for the preparation of (+)-11β,13-dihydrocostunolide[22] (**4**), (+)-1,10-epoxy-11β,13-dihydrocostunolide[14a] (**5**), and the key intermediates **6** and **7**.[5]

5.2. Synthesis of ketone 8

Water (100 μL) and Dess–Martin periodinane (DMP) (1.84 g, 4.3 mmol) were added to a solution of compound **6** (900 mg, 3.6 mmol) in CH_2Cl_2 (125 mL). The mixture was stirred at rt for 1 h, diluted with CH_2Cl_2 and washed with a 1:1 mixture (50 mL) of saturated solutions of $NaHCO_3$ and $Na_2S_2O_3$, and with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed. The residue was submitted to flash chromatography (*t*-BuOMe) to provide ketone **8** (85 mg, 95%). IR (film) ν_{max} 1767, 1703 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.04 (t, J=10.3 Hz, 1H), 2.73 (ddd, J=15.6, 11.7, 5.5 Hz, 1H), 2.37 (m, 1H), 2.25 (dt, J=15.0, 5.3 Hz, 1H), 2.10–1.75 (m, 5H), 1.60–1.40 (m, 4H), 1.26 (s, 3H), 1.21 (d, J=7.7 Hz, 3H), 1.21 (d, J=5.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 214.2 (C), 179.2 (C), 79.4 (CH), 52.9 (CH), 49.9 (CH), 41.6 (CH), 36.9 (C), 34.9 (CH_2), 34.4 (CH_2), 32.5 (CH_2), 26.9 (CH), 22.7 (CH_2), 20.3 (CH_3), 15.4 (CH_3), 12.5 (CH_3). HRMS (FABMS): m/z 273.1460 $[M+Na]^+$, calcd for $C_{15}H_{22}O_3Na$: 273.1466.

5.3. Synthesis of alkene 9

A sample of *p*-toluenesulfonyl hydrazide (290 mg, 1.85 mmol) was added to a solution of **8** (420 mg, 1.68 mmol) in MeOH (10 mL). The mixture was stirred under reflux for 30 min and at 25 °C for 14 h. The solvent was then removed and the residue dissolved in *t*-BuOMe and washed with brine. The organic layer was dried with anhyd Na_2SO_4, and the solvent removed to give the hydrazone intermediate. To this intermediate (130 mg, 0.36 mmol) dissolved in anhyd toluene (28 mL), NaH (260 mg, 10.8 mmol) was added under an argon atmosphere. The mixture was stirred at 90 °C for 2 h, diluted with *t*-BuOMe (50 mL) and washed with brine. The organic layer was dried over anhyd Na_2SO_4, the solvent was removed and the residue was submitted to flash chromatography (*t*-BuOMe/hexane 1:4) to give alkene **9** (50 mg, 50% after two steps). IR (film) ν_{max} 2936, 1768 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.04 (s, 2H), 4.01 (dd, J=11.5, 9.7 Hz, 1H), 2.40–1.20 (m, 10H), 1.19 (d, J=6.9 Hz, 3H), 1.08 (s, 3H), 1.04 (d, J=6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 179.8 (C), 136.9 (CH), 123.4 (CH), 80.2 (CH), 54.1 (CH), 49.2 (CH), 41.9 (CH), 41.2 (CH_2), 36.9 (C), 32.4 (CH_2), 25.5 (CH), 23.4 (CH_2), 22.2 (CH_3), 17.2 (CH_3), 12.6 (CH_3). HRMS (FABMS): m/z 257.1516 $[M+Na]^+$, calcd for $C_{15}H_{22}O_2Na$: 257.1517.

5.4. Synthesis of alcohol 10

Under an argon atmosphere, SeO_2 (72 mg, 0.63 mmol) was added to a solution of **9** (50 mg, 0.21 mmol) in anhyd dioxane (10 mL). The mixture was stirred at 100 °C for 3 h, diluted with *t*-BuOMe and washed with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed. The residue was submitted to flash chromatography (*t*-BuOMe/hexane 1:1) to provide alcohol **10** (35 mg, 70%). IR film ν_{max} 3418, 1768 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.70 (d, J=10 Hz, 1H), 5.63 (dd, J=10.0, 4.0 Hz, 1H), 3.98 (dd, J=11.1, 10.0 Hz, 1H), 3.85 (d, J=3.8 Hz, 1H), 2.30 (m, 2H), 2.18 (br s, 1H(OH)), 2.01 (dd, J=11.7, 4.4 Hz, 1H), 1.81 (m, 1H), 1.70–1.20 (m, 4H), 1.12 (d, J=6.8 Hz, 3H), 1.01 (s, 3H), 0.97 (d, J=7.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 179.9 (C), 140.7 (CH), 124.8 (CH), 79.6 (CH), 69.9 (CH), 54.0 (CH), 44.5 (CH), 41.8 (CH), 40.4 (CH_2), 37.1 (C), 35.7 (CH), 23.2 (CH_2), 21.8 (CH_3), 14.8 (CH_3), 12.6 (CH_3). HRMS (FABMS): m/z 273.1463 $[M+Na]^+$, calcd for $C_{15}H_{22}O_3Na$: 273.1466.

5.5. Synthesis of conjugated lactone 11

A solution of compound **10** (30 mg, 0.12 mmol) in anhyd THF (3 mL) was cooled until −78 °C under argon. KHMDS (192 mg, 0.48 mmol) was added and the mixture was stirred at −78 °C for 30 min. Then, PhSeCl (93 mg, 0.42 mmol) was added and the mixture was stirred at −78 °C for 2 h. The mixture was diluted with *t*-BuOMe and washed with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed under vacuum. The residue was dissolved in CH_2Cl_2 (6 mL) and pyridine (1 mL) and H_2O_2 (1 mL, 30% w/w) were added. The mixture was heated under reflux for 10 min, diluted with CH_2Cl_2 and washed with a 10% HCl solution (10 mL) and brine. The organic layer was dried over anhyd Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography (*t*-BuOMe/hexane 3:7) to afford **11** (17 mg, 58%). IR (film) ν_{max} 3423, 1766 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.04 (d, J=3.1 Hz, 1H), 5.74 (d, J=10.0 Hz, 1H), 5.67 (dd, J=10.0, 4.2 Hz, 1H),

5.37 (d, J=3.1 Hz, 1H), 3.98 (t, J=11.0 Hz, 1H), 3.89 (d, J=4.0 Hz, 1H), 2.55 (tq, J=11.1, 3.2 Hz, 1H), 2.36 (m, 1H), 2.16 (dd, J=11.7, 4.4 Hz, 1H), 2.03 (dq, J=13.3, 3.6 Hz, 1H), 1.53 (m, 3H), 1.03 (s, 3H), 1.01 (d, J=7.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 171.0 (C), 140.6 (CH), 139.9 (C), 124.8 (CH), 116.6 (CH_2), 79.9 (CH), 69.9 (CH), 51.4 (CH), 45.0 (CH), 40.1 (CH_2), 37.3 (C), 35.6 (CH), 21.8 (CH_2), 21.6 (CH_3), 14.9 (CH_3). HRMS (FABMS): m/z 271.1313 $[M+Na]^+$, calcd for $C_{15}H_{20}O_3Na$: 271.1310.

5.6. Synthesis of conjugated ketone 12

DMP (103 mg, 0.24 mmol) was added to a solution of **11** (50 mg, 0.20 mmol) in CH_2Cl_2/H_2O (10 mL/10 μL). The mixture was stirred at rt for 1.5 h, diluted with CH_2Cl_2 and washed with a 1:1 mixture (20 mL) of saturated solutions of $NaHCO_3$ and $Na_2S_2O_3$, and with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed. The residue was purified by flash chromatography (*t*-BuOMe/hexane 3:2) to afford ketone **12** (45 mg, 90%). IR film ν_{max} 1768, 1668 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.78 (d, J=10.0 Hz, 1H), 6.10 (d, J=3.2 Hz, 1H), 5.92 (d, J=10.1 Hz, 1H), 5.43 (d, J=3.0 Hz, 1H), 4.03 (t, J=11.1 Hz, 1H), 2.86 (quint, J=7.5 Hz, 1H), 2.54 (tq, J=14.0, 3.1 Hz, 1H), 2.43 (dd, J=11.6, 6.4 Hz, 1H), 2.12 (m, 1H), 1.73–1.55 (m, 3H), 1.29 (d, J=7.8 Hz, 3H), 1.23 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 201.3 (C), 170.3 (C), 158.2 (CH), 139.0 (C), 127.1 (CH), 117.4 (CH_2), 79.0 (CH), 50.5 (CH), 48.6 (CH), 40.2 (CH), 38.8 (CH_2), 38.2 (C), 22.7 (CH_3), 21.4 (CH_2), 13.7 (CH_3). HRMS (FABMS): m/z 269.1156 $[M+Na]^+$, calcd for $C_{15}H_{18}O_3Na$: 269.1153.

5.7. Synthesis of (+)-tuberiferine (1)

DBU (0.24 mL) was added to a solution of **12** (40 mg, 0.16 mmol) in anhyd toluene (8 mL) under an argon atmosphere. The mixture was stirred at 60 °C for 1.5 h and at 25 °C for 10 h. The mixture was diluted with CH_2Cl_2 and washed with a 10% HCl solution (20 mL) and brine. The organic layer was dried over anhyd Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography (*t*-BuOMe/hexane 3:2) to afford **1** (37 mg, 92%). Spectroscopic properties, including IR and NMR data, and optical rotation were in agreement with those previously described for (+)-tuberiferine.[18,20c]

5.8. Synthesis of ketone 13

A sample of DMP (920 mg, 2.15 mmol) was added to a solution of **7** (450 mg, 1.8 mmol) in CH_2Cl_2/H_2O (70 mL/50 μL). The mixture was stirred at rt for 1 h, diluted with CH_2Cl_2 and washed with a 1:1 mixture (30 mL) of saturated solutions of $NaHCO_3$ and $Na_2S_2O_3$, and with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed. The residue was submitted to flash chromatography (*t*-BuOMe) to give ketone **13** (400 mg, 89%). IR film ν_{max} 2936, 1782, 1708 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 (s, 1H), 5.08 (s, 1H), 4.12 (t, J=10.5 Hz, 1H), 2.74–2.57 (m, 2H), 2.46–2.30 (m, 4H), 1.95 (dq, J=12.9, 3.0 Hz, 1H), 1.85 (dt, J=14.4, 3.2 Hz, 1H), 1.75 (td, J=12.9, 4.2 Hz, 1H), 1.60 (m, 1H), 1.47 (td, J=12.6, 3.8 Hz, 1H), 1.24 (d, J=6.9 Hz, 3H), 1.12 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, DEPT): δ 212.0 (C), 178.9 (C), 140.8 (C), 112.6 (CH_2), 78.6 (CH), 52.2 (CH), 51.8 (CH), 50.2 (C), 41.0 (CH), 37.6 (CH_2), 34.0 (CH_2), 31.5 (CH_2), 22.5 (CH_2), 18.1 (CH_3), 12.4 (CH_3). HRMS (FABMS): m/z 271.1307 $[M+Na]^+$, calcd for $C_{15}H_{20}O_3Na$: 271.1310.

5.9. Synthesis of diene 14

A sample of *p*-toluenesulfonyl hydrazide (193 mg, 1.23 mmol) was added to a solution of **13** (280 mg, 1.12 mmol) in MeOH (10 mL). The mixture was stirred under reflux for 30 min and, subsequently, at 25 °C for 14 h. The solvent was removed and the residue was dissolved in *t*-BuOMe and washed with brine. The organic layer was dried with anhyd Na_2SO_4 and the solvent was removed to give the corresponding hydrazone intermediate. NaH (260 mg, 10.8 mmol) was added to this intermediate (130 mg, 0.36 mmol) in anhyd toluene (28 mL) under an argon atmosphere. The mixture was stirred at 90 °C for 2 h, diluted with *t*-BuOMe (50 mL) and washed with brine. The organic layer was dried over anhyd Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography (*t*-BuOMe/hexane 1:4) to provide diene **14** (47 mg, 47% after two steps). IR film ν_{max} 1770 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.52 (s, 2H), 5.02 (br s, 1H), 4.89 (s, 1H), 4.07 (dd, J=10.9, 10.4 Hz, 1H), 2.94–2.69 (m, 2H), 2.39 (d, J=11.7 Hz, 1H), 2.34 (dq, J=13.6, 6.9 Hz, 1H), 1.87–1.43 (m, 5H), 1.22 (d, J=6.8 Hz, 3H), 0.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 179.4 (C), 141.4 (C), 137.2 (CH), 123.9 (CH), 109.4 (CH_2), 79.3 (CH), 52.8 (CH), 52.3 (CH), 41.2 (CH), 39.4 (C), 37.5 (CH_2), 34.8 (CH_2), 23.2 (CH_2), 20.8 (CH_3), 12.5 (CH_3). HRMS (FABMS): m/z 255.1358 $[M+Na]^+$, calcd for $C_{15}H_{20}O_2Na$: 255.1361.

5.10. Synthesis of alcohol 15

To a solution of **14** (80 mg, 0.34 mmol) in CH_2Cl_2 (10 mL), SeO_2 (20 mg, 0.17 mmol) and *t*-BuOOH (0.28 mL, 6 M in decane) were added. The mixture was stirred at 25 °C for 4 h, diluted with CH_2Cl_2 (20 mL) and washed with brine. The organic layer was dried over anhyd Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography (*t*-BuOMe/hexane 1:1) to give alcohol **15** (56 mg, 75%). IR film ν_{max} 3421, 1768 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.72 (d, J=9.8 Hz, 1H), 5.66 (dd, J=9.8, 3.5 Hz, 1H), 5.27 (s, 1H), 5.08 (s, 1H), 4.38 (d, J=3.3 Hz, 1H), 4.05 (t, J=10.5 Hz, 1H), 2.66 (d, J=11.0 Hz, 1H), 2.33 (dq, J=13.5, 6.8 Hz, 1H), 1.98 (s, 1H(OH)), 1.88–1.50 (m, 5H), 1.20 (d, J=6.8 Hz, 3H), 0.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 179.3 (C), 145.1 (C), 141.5 (CH), 125.4 (CH), 115.6 (CH_2), 78.7 (CH), 69.1 (CH), 52.7 (CH), 48.2 (CH), 41.2 (CH), 40.0 (C), 36.9 (CH_2), 23.0 (CH_2), 19.5 (CH_3), 12.5 (CH_3). HRMS (FABMS): m/z 271.1304 $[M+Na]^+$, calcd for $C_{15}H_{20}O_3Na$: 271.1310.

5.11. Synthesis of α,β-unsaturated lactone 16

Compound **15** (90 mg, 0.36 mmol) was dissolved in anhyd THF (9 mL) under an argon atmosphere and the solution was cooled at −78 °C. KHMDS (504 mg, 2.52 mmol) was added to this solution and the mixture was stirred at −78 °C for 30 min. Then, PhSeCl (242 mg, 1.26 mmol) was added and the mixture was stirred at −78 °C for 2 h. The mixture was diluted with *t*-BuOMe and washed with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed under vacuum. The residue was dissolved in CH_2Cl_2 (12 mL), and pyridine (1 mL) and H_2O_2 (1 mL, 30% w/w) were added. The mixture was heated under reflux for 10 min, diluted with CH_2Cl_2 and washed with a 10% HCl solution (10 mL) and brine. The organic layer was dried over anhyd Na_2SO_4, the solvent was removed and the residue was purified by flash chromatography (*t*-BuOMe/hexane 3:7) to afford **16** (45 mg, 50%). IR film ν_{max} 3402, 1763 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.09 (d, J=2.4 Hz, 1H), 5.76 (d, J=9.9 Hz, 1H), 5.70 (dd, J=9.9, 3.2 Hz, 1H), 5.41 (d, J=2.3 Hz, 1H), 5.32 (s, 1H), 5.15 (s, 1H), 4.43 (d, J=3.2 Hz, 1H), 4.05 (t, J=10.9 Hz, 1H), 2.81 (d, J=11.1 Hz, 1H), 2.62 (m, 1H), 2.08 (m, 1H), 1.75–1.60 (m, 3H), 0.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, DEPT): δ 171.6 (C), 144.8 (C), 141.4 (CH), 139.1 (C), 125.4 (CH), 117.1 (CH_2), 114.0 (CH_2), 78.9 (CH), 69.2 (CH), 50.0 (CH), 48.6 (CH), 40.2 (C), 36.7 (CH_2), 21.4 (CH_2), 19.5 (CH_3). HRMS (FABMS) m/z 269.1153 $[M+Na]^+$, calcd for $C_{15}H_{18}O_3Na$: 269.1153.

5.12. Synthesis of (+)-dehydrobrachylaenolide (2)

Water (10 μL) and DMP (72 mg, 0.16 mmol) were added to a solution of compound **16** (30 mg, 0.12 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at rt for 1.5 h, diluted with CH_2Cl_2 and washed

with a 1:1 mixture (10 mL) of saturated solutions of $NaHCO_3$ and $Na_2S_2O_3$, and with brine. The organic layer was dried over anhyd Na_2SO_4 and the solvent removed. The residue was submitted to flash chromatography (*t*-BuOMe/hexane 1:1) to give **2** (25 mg, 84%). Optical rotation, ^1H, and ^{13}C NMR data of **2** matched those previously described.[19,21]

5.13. Antifungal analysis

Freshly harvested spores of the NRRL1555 wild strain of the filamentous fungus *P. blakesleeanus*[28b] were inoculated (7×10^5 spores/L) in several 250 mL Erlenmeyer flasks, each containing 50 mL of sterile minimal medium.[29] Immediately afterwards, THF solutions (1 mL) with different concentrations of products **1** and **2** and amphotericin B were added in order to reach effective concentrations in the media ranging from 100 to 3 μg/mL. The cultures were incubated at 23 °C with orbital shaking (200 rpm), under the light of four 40 W fluorescent lamps, for 7 days. The cultures were then filtered and the mycelia were lyophilized, weighted and compared with a control culture incubated following the same procedure (including THF addition) but without any antifungal agent. All the experiments were performed in duplicate and the results given herein correspond to the arithmetical media of weights measured in each case.

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