Microbial Transformation of Sesquiterpene Lactones by the Fungi *Cunninghamella echinulata* and *Rhizopus oryzae*

Alejandro F. Barrero,*,† J. Enrique Oltra,† Délio S. Raslan,‡ and Dênia A. Saúde‡

Departamento de Química Orgánica, Instituto de Biotecnología, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071-Granada, Spain, and Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, MG, Brazil

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Incubations of the fungi *Cunninghamella echinulata* and *Rhizopus oryzae* with the sesquiterpene lactones (+)-costunolide (**1**), (+)-cnicin (**2**), (+)-salonitenolide (**3**), (−)-dehydrocostuslactone (**4**), (−)-lychnopholide (**5**), and (−)-eremantholide C (**6**) were perfomed. Incubation of **1** with *C. echinulata* afforded $\Delta^{11(13)}$-dihydrogenation and $\Delta^{1(10)}$-epoxidation products (**7**−**10**). *C. echinulata* also hydrolyzed the side chain of **2**, and transformed **4** into (+)-11α,13-dihydrodehydrocostuslactone (**12**), a new natural product. *R. oryzae* converted **4** into both $\Delta^{11(13)}$-dihydrogenation and $\Delta^{10(14)}$-epoxidation products (**16** and **17**). Both fungi transformed **5** into (−)-16-(1-methyl-1-propenyl)eremantholanolide (**13**), providing experimental evidence for the biosynthesis of the eremantholide hemiketal unit. Compounds **3** and **6** were not metabolized by either fungus under the test conditions.

As part of a program directed toward the enantiospecific synthesis of natural sesquiterpene lactones with biological activity,[1,2] a microbiological alternative to improve the regioselectivity and stereoselectivity of some chemical reactions was considered. However, apart from biological degradation of α-santonin, scant information about bioconversion of other sesquiterpene lactones was available.[3] Therefore, microbiological transformation of some accessible sesquiterpenoids (**1**−**6**), which can be useful as raw materials for enantiospecific synthesis of bioactive lactones, was investigated using the fungi *Cunninghamella echinulata* and *Rhizopus oryzae*. The results from incubations with (+)-costunolide[4] (**1**), (+)-cnicin[5] (**2**), (−)-dehydrocostuslactone[6] (**4**), and (−)-lychnopholide[7] (**5**) are reported in this paper. (+)-Salonitenolide[5] (**3**) and (−)-eremantholide C[7] (**6**) were not transformed by either organism.

Results and Discussion

Incubations with *C. echinulata* were performed by adding the exogenous substrate to a 24- or a 48-h culture. When (+)-costunolide (**1**) was added to a 24-h culture, strong inhibition of mycelium growth was observed. Therefore, a second incubation was carried out using a more developed biomass (48 h). Under 24-h. conditions, costunolide was not completely transformed, but a hydrogenation product, identified as (+)-11β,13-dihydrocostunolide[4] (**7**), was isolated. Incubation of **1** with 48-h biomass led to three oxidation products, the well-known 1β-hydroxyeudesmanolides (+)-santamarine[8] (**8**), (+)-reynosin[9] (**9**), and (+)-1β-hydroxyarbusculin A[10] (**10**). Absolute stereochemistry of (+)-costunolide (**1**) has been established.[11] Therefore, transformation of **1** into **8**−**10** indicates that the absolute configuration of (+)-santamarine, (+)-reynosin, and (+)-1β-hydroxyarbusculin A is as shown. Formation of **8**−**10** can be explained by enzymatic epoxidation of **1** to 1β,10α-epoxicostunolide (**11**), and subsequent electrophilic opening of the epoxide with concomitant rearrangement to the eudesmanolide skeleton, as presumably occurs in plant biogenesis of 1β-hydroxyeudesmanolides.[12]

Incubation of *C. echinulata* with (+)-cnicin (**2**) afforded (+)-salonitenolide[5] (**3**). No hydrogenation products or mycelium growth inhibition were observed. Selective hydrolysis of the (+)-cnicin side chain to (+)-salonitenolide is of synthetic interest because **2** is a widespread metabolite, abundant in several species of the *Centaurea* genus[5] as well as in *Cnicus benedictus*,[13] and **3** is a raw material useful for the synthesis of (+)-stoebenolide,[2] (+)-dehydromelitensin,[2] and (+)-vernolepin derivatives.[1]

When (−)-dehydrocostuslactone (**4**) was added to a 24-h culture of *C. echinulata*, complete inhibition of the mycelium growth was observed. Compound **4** was recovered unchanged after the incubation period. However, incubation of a more developed biomass (48 h) with **4** provided a guaianolide not previously described and identified as (+)-11α,13-dihydrodehydrocostuslactone (**12**). In the ^{1}H NMR spectrum, a methyl doublet assignable to H-13 and signals for only four olefinic protons were evident, indicating saturation of the $\Delta^{11(13)}$ double bond of **4**. The coupling constant between H-11 and H-7 (J = 7.8 Hz) indicated the β arrangement of the C-13 methyl group of **12**. The NOE observed between H-13 and H-6 confirmed this stereochemistry.

Incubation of a 24-h mycelium of *C. echinulata* with (−)-lychnopholide (**5**) afforded an eremantholide identified as (−)-16-(1-methyl-1-propenyl)eremantholanolide[14] (**13**). Biogenesis of **13** in plants, by reductive cyclization of the corresponding 8α-angelate, was proposed previously,[14,15] along the same line of an earlier hypothesis about the biosynthesis of closely related structures called eremantholides A and B.[16] However, apart from the joint occurrence of **5** and **13** in plants of the *Lychnophora*, *Piptolepis*, and *Proteopsis* genera,[17] no other evidence confirming the biogenesis of **13** has been reported so far. Transformation of **5** into **13** by *Cunninghamella* lends experimental support to the above-mentioned biogenetic hypothesis. To obtain information on the mechanism involved in the intramolecular cyclization to the hemiketal unit contained in **13**, chemical treatment of **5** with hydrides was tested. Reaction with $NaBH_4$ gave the alcohol **14**, and treatment with Bu_3SnH only causes isomerization of the lateral chain, leading to **15**.[18] Formation of eremantholide **13** was not detected in any case. Despite chemical hydride failure to mimic the

* To whom correspondence should be addressed. Tel. 34 958 24 33 18. Fax: 34 958 24 84 37. E-mail: afbarre@goliat.ugr.es.
† Universidad de Granada, Spain.
‡ Universidade Federal de Minas Gerais, Brazil.

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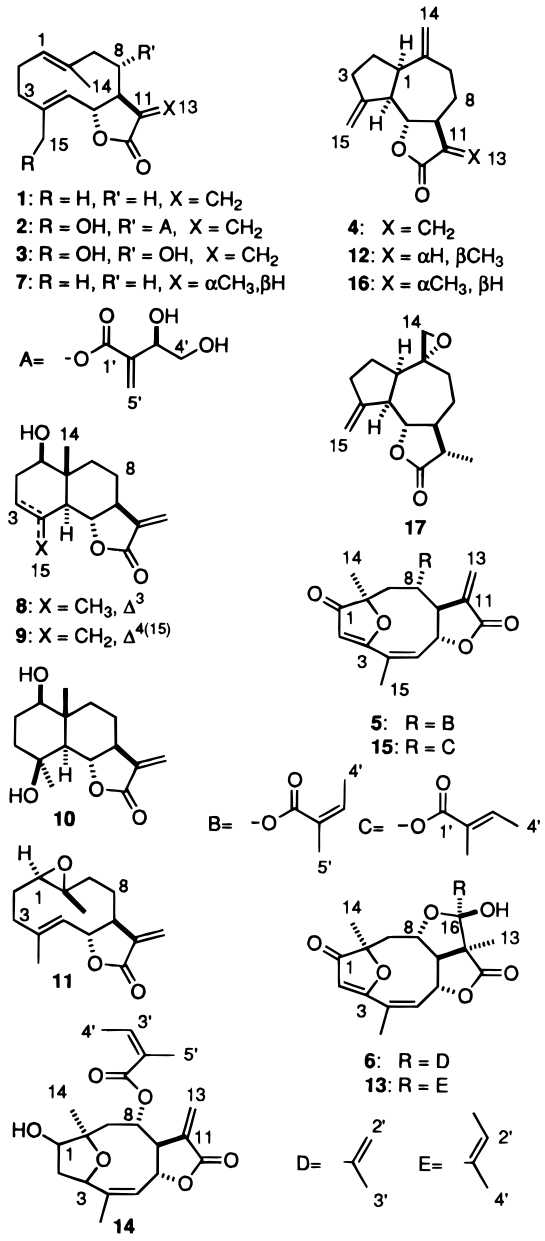
enzymatic cyclization to the hemiketal unit of **13**, the hydrogenating ability toward the α-methylene-γ-lactone group, showed by the 24-h mycelium of *Cunninghamella*, must be involved in the biogenesis of **13**. In this way, when **5** was added to a 48-h mycelium (which lacks any hydrogenating ability), no biotransformation products were detected after incubation, and the exogenous substrate was recovered unchanged.

Incubations of *Rhizopus oryzae* with lactones **4** and **5** were carried out by adding each lactone to a 48-h culture. Microbial transformation of (−)-dehydrocostuslactone (**4**) afforded two products identified as (+)-11*β*,13-dihydrodehydrocostuslactone (mokko lactone, **16**)[19] and (+)-11*β*,13-dihydro-10,14-epoxydehydrocostuslactone (**17**). High-resolution NMR data for **16** are included in the Experimental Section, to facilitate further differentiation from its C-11 epimer (**12**). Previously, (−)-dehydrocostuslactone (**4**) and (+)-mokko lactone (**16**) were chemically correlated with (−)-α-santonin,[20] whose absolute stereochemistry was determined by X-ray analysis.[21] Thus, the absolute configuration of **17** was established as shown. Compound **17** was previously found in the medicinal plant *Vladimiria souliei*, and its structure was proposed on the basis of ^{1}H NMR data.[22] Biological synthesis of **17**, from **4**, confirms its structure and the absolute stereochemistry of the (+)-enantiomer. Unfortunately, optical rotation of the *V. souliei* metabolite was not reported. For synthetic purposes, *C. echinulata* and *R. oryzae* constitute a fungal pair useful for selecting a given stereochemistry in $\Delta^{11(13)}$-hydrogenation processes on **4** and, presumably, other guaianolides. Microbial transformation of lychnopholide (**5**) by *R. oryzae*, afforded the same eremantholide (**13**) isolated from the *Cunninghamella* culture.

Epoxidation reactions and hydrogenation of the C-11−C-13 double bond seem to be the most common processes in microbial transformation of sesquiterpene lactones by the filamentous fungi *C. echinulata* and *R. oryzae*. Cytotoxic activity of sesquiterpene lactones lies chiefly in their α-methylene-γ-lactone group.[23] Hence, regioselective hydrogenation at $\Delta^{11(13)}$ might be a defense mechanism of these organisms against the fungistatic activity of some lactones. From both the biochemical and the synthetic points of view, cyclization to the hemiketal unit of eremantholides is the most important feature of the biotransformations presented in this paper, due to its biogenetic significance and the difficulties encountered achieving it by chemical methods.



Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin−Elmer 141 polarimeter. IR spectra were obtained as liquid films, between NaCl plates, on a 983 G Perkin−Elmer apparatus. NMR spectra were recorded on Bruker AM 300 and Bruker ARX 400 spectrometers. Chemical shifts are reported in parts per million (*δ*) relative to TMS, and coupling constants (*J*) are in Hertz. ^{13}C NMR assignments are tentative unless otherwise stated. Carbon substitution degrees were established by DEPT multipulse sequence. LRMS were determined on a 5988A Hewlett−Packard instrument, and HRMS were measured on an Autospec-Q VG-Analytical (FISONS) mass spectrometer. Thin-layer chromatography (TLC) was performed on precoated 0.25-mm thick Merck plates of Si gel 60 F_{254}, using a 7% phosphomolybdic acid solution (EtOH) to visualize spots. Column chomatography was carried out on Si gel, eluting with hexane−*t*-BuOMe mixtures of increasing polarity, unless otherwise stated; and flash chromatography was performed as described previously.[24] Reagents were purchased from Aldrich, Merck, or Fluka and were used as received. All solvents were purified and dried following standard procedures.[25]

Sesquiterpene Lactones. Lactones **1** and **4** were isolated from Costus Resinoid, a commercially available extract from costus roots (*Saussurea lappa*), purchased from Pierre Chauvet S. A., Seillans, France. The extract (5 g) was column chromatographed over 5% $AgNO_3$−Si gel (150 g). Elution yielded **4** (0.92 g, hexane−*t*-BuOMe, 80:20) and **1** (0.47 g, hexane−*t*-BuOMe, 60:40). Cnicin (**2**) was obtained from *Centaurea malacitana*, as previously described.[5] Salonitenolide (**3**) was obtained by selective saponification of the side chain of **2**.[5] Compounds **5** and **6** were isolated from aerial parts of *Lychnophora trichocarpa*.[7] Physical data of these lactones, including optical rotation, were consistent with published data.

Microorganisms and Culture Media. *Cunninghamella echinulata* (NRRL3655) and *Rhizopus oryzae* (ATCC11145) were purchased from Colección Española de Cultivos Tipo (CECT), Universidad de Valencia, Valencia (Spain). Stock cultures of *C. echinulata* were stored on agar slants (potato dextrose agar plus 2% yeast extract). *R. oryzae* was mantained on agar slants prepared with a mixture of glucose (20 g), L-asparagin·H_2O (2 g), KH_2PO_4 (5 g), $MgSO_4$·$7H_2O$ (0.5 g), $CaCl_2$ (0.028 g), tiamine·HCl (0.001 g), citric acid·H_2O (0.002

g), $Fe(NO_3)_3 \cdot 9H_2O$ (0.0015 g), $ZnSO_4 \cdot 7H_2O$ (0.001 g), $MnSO_4 \cdot H_2O$ (300 μg), $CuSO_4 \cdot 5H_2O$ (50 μg), $Na_2MoO_4 \cdot 2H_2O$ (50 μg), yeast extract (1 g), and agar (15 g) in tap water, q. s. 1 L. Liquid culture medium for *C. echinulata* contained glucose (20 g), yeast extract (5 g), peptone (5 g), NaCl (5 g), and K_2HPO_4 (5 g) in 1 L of distilled H_2O. The culture medium used for *R. oryzae* was the broth stock medium.

Incubations with *Cunninghamella echinulata*. To prepare a homogeneous suspension of biomass, *C. echinulata* was first grown in two 500-mL culture flasks, each containing 100 mL of liquid medium, at 26 °C, on a rotatory shaker (200 rpm), for 48 h. The biomass in suspension obtained was innoculated (2 mL per flask) in several 500-mL flasks, each containing 100 mL of fresh liquid medium. The inoculated flasks were submitted to a first stage of incubation on a rotatory shaker (200 rpm), at 26 °C, either for 24 h (condition A) or for 48 h (condition B). After the first stage, the exogenous substrates were evenly distributed (10 mg in 0.2 mL of THF per flask) among the 24- and/or 48-h-old cultures, and incubated at 26 °C, on a rotatory shaker (200 rpm), for an additional time (second stage of incubation). During the second stage, aliquots from cultures were taken daily and analyzed by TLC and ^{1}H NMR in order to determine the degree of transformation of substrates. In all experiments, two control flasks without biomass (for substrate stability) and two flasks without exogenous substrate (for endogenous metabolites) were used.

Biotransformation of Costunolide (1). (+)-Costunolide (200 mg) was evenly distributed among 20 flasks containing 24-h-old first-stage biomass. Incubation was stopped after 78 h of the second stage. Culture broth was filtered, and the filtrate was extracted with EtOAc. The organic solvent was removed, and the residue (138 mg) was chromatographed on Si gel. Compounds **1** (34 mg, hexane−*t*-BuOMe 80:20) and **7** (6 mg, hexane−*t*-BuOMe 80:20) were obtained. Another 200 mg of **1** was dissolved in THF (4 mL) and evenly distributed among 20 flasks containing 48-h-old first-stage biomass. Incubation was stopped after 54 h, and the organic metabolites were extracted from the medium. Column chromatography of the organic extract (190 mg) afforded **8** (9 mg, hexane−*t*-BuOMe 80:20), **9** (15 mg, hexane−*t*-BuOMe 70:30), and **10** (21 mg, hexane−*t*-BuOMe 30:70).

Biotransformation of Cnicin (2). Incubation of (+)-cnicin (200 mg) with 24-h-old biomass was carried out as described above for costunolide. Biotransformation was stopped after 54 h. Column chromatography of the organic extract (181 mg) yielded **3** (11 mg, $CHCl_3$−Me_2CO 80:20).

Biotransformation of Dehydrocostuslactone (4). (−)-Dehydrocostuslactone (220 mg) was evenly distributed between two flasks containing 24-h-old biomass (condition A) and 20 flasks containing 48-h-old biomass (condition B). Biotransformations were stopped either after 4 days (condition A) or after 2 days (condition B). Then, organic extracts A (35 mg) and B (400 mg) were obtained. ^{1}H NMR spectrum and TLC analysis showed that extract A was mainly formed by compound **4**. Column chromatography of extract B afforded **4** (29 mg, hexane−*t*-BuOMe 90:10) and **12** (6 mg, hexane−*t*-BuOMe 85:15).

Compound 12: oil; $[\alpha]^{25}_D$ +13.7° (*c* 0.32, $CHCl_3$); IR (film) ν_{max} 1773 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.19 (1H, d, J = 2.1 Hz, H -15a), 5.05 (1H, d, J = 2.0 Hz, H-15b), 4.86 (1H, br s, H-14a), 4.77 (1H, br s, H-14b), 4.02 (1H, t, J = 9.6 Hz, H-6), 2.86 (2H, m, H-1, H-5), 2.67 (1H, dq, J = 7.8, 7.8 Hz, H-11), 2.49 (1H, br dt, J = 12.0, 3.0 Hz, H-9β), 1.17 (3H, d, J = 7.8 Hz, H-13); NOE-dif experiments, proton irradiated (NOEs observed) H-15a (H-15b, H-6), H-15b (H-15a), H-14a (H-14b, H-9β), H-14b (H-14a), H-6 (H-15a, H-13), H-13 (H-11, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.0 (s, C-4), 150.0 (s, C-10), 111.8 (t, C-14), 109.5 (t, C-15), 85.3 (d, C-6), 52.3 (d, C-7), 47.2 (d, C-1), 45.0 (d, C-5), 39.5 (d, C-11), 37.6 (t, C-8), 32.6 (t, C-9), 29.8 (t, C-2), 28.9 (t, C-3), 11.5 (q, C-13); CIMS *m*/*z* 233 [M + H]$^+$ (41), 187 (35), 159 (100), 157 (17), 81 (21), 57 (24), 55 (22), 43 (36); HRFABMS *m*/*z* 255.1361 (calcd for $C_{15}H_{20}O_2Na$, 255.1361).

Biotransformation of Lychnopholide (5). (−)-Lychnopholide (430 mg) was evenly distributed among 23 flasks containing 24-h-old biomass (A) and 20 flasks containing 48-h biomass (B). Biotransformations were stopped either after 4 days (A) or after 64 h (B). Extracts A (289 mg) and B (365 mg) were obtained. Column chromatography of extract A afforded **5** (27 mg, hexane−EtOAc 85:15) and eremantholide **13** (14 mg, hexane−EtOAc 85:15). Column chromatography of extract B yielded 9 mg of **5**, and no eremantholides were detected.

Compound 13: ^{1}H NMR data matched those described by Zdero et al.;[14] ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.6 (s, C-1), 187.2 (s, C-3), 176.0 (s, C-12), 175.8 (s, C-1′), 134.8 (d, C-5), 130.1 (s, C-4), 127.9 (d, C-2′), 108.1 (s, C-16), 104.6 (d, C-2), 90.2 (s, C-10), 81.5 (d, C-6), 78.9 (d, C-8), 62.0 (d, C-7), 61.3 (s, C-11), 43.7 (t, C-9), 22.0 (q, C-4′), 21.4 (q, C-13), 20.6 (q, C-14), 20.4 (q, C-15), 15.4 (q, C-3′).

Reduction of 5 with $NaBH_4$. Lychnopholide (**5**) (200 mg, 0.56 mmol), in 5 mL of MeOH, was chilled down to 0 °C, and $NaBH_4$ was added. The solution was stirred at 25 °C for 1.5 h after the hydride addition. Then, 20 mL of H_2O were added, and the mixture was neutralized with AcOH and extracted with EtOAc. Flash chromatography (hexane−EtOAc 70:30) of the organic extract afforded 30 mg of **14** as a gum: $[\alpha]^{25}_D$ −22.6° (*c* 1.83, $CHCl_3$); IR (film) ν_{max} 3426, 1763, 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.22 (1H, dd, J = 2.4, 0.8 Hz, H-13a), 6.16 (1H, qq, J = 7.2, 1.5 Hz, H-3′), 5.65 (1H, dd, J = 2.1, 0.8 Hz, H-13b), 5.56 (1H, dq, J = 4.4, 1.7 Hz, H-5), 5.37 (1H, m, H-6), 5.18 (1H, dt, J = 10.6, 3.5 Hz, H-8), 4.38 (1H, dd, J = 10.7, 6.4 Hz, H-3), 4.16 (1H, m, H-7), 4.12 (1H, dd, J = 9.4, 6.4, H-1), 2.54 (1H, dt, J = 12.7, 6.4 Hz, H-2), 2.27 (1H, dd, J = 16.3, 3.5 Hz, H-9), 2.02 (3H, dq, J = 7.2, 1.5 Hz, H-4′), 1.96 (3H, quin, J = 1.5 Hz, H-5′), 1.75 (3H, t, J = 1.7 Hz, H-15), 1.40 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2 (s, C-12), 166.9 (s, C1′), 140.0 (d, C-3′), 139.3 (s, C-4), 138.0 (s, C-11), 127.4 (d, C-5), 127.1 (s, C-2′), 124.5 (t, C-13), 82.5 (s, C-10), 79.7 (d, C-1), 77.2 (d, C-3), 76.6 (d, C-6), 74.3 (d, C-8), 49.3 (d, C-7), 37.7 (t, C-2), 34.4 (t, C-9), 27.7 (q, C-14), 23.9 (q, C-15), 20.7 (q, C-5′), 15.9 (q, C-14); NMR data were assigned with the aid of 2D NMR experiments ^{1}H−^{1}H homonuclear correlation (COSY), ^{1}H−^{13}C direct (HETCOR), and long-range (HMBC) heteronuclear correlations; CIMS *m*/*z* 363 [M + H]$^+$ (1), 263 (3), 101 (81), 83 (50), 69 (28), 55 (51), 43 (100); HRFABMS *m*/*z* 385.1628 (calcd for $C_{20}H_{26}O_6Na$, 385.1627).

Treatment of 5 with Bu_3SnH. A solution of Bu_3SnH (0.09 mL, 0.031 mmol) and 2,2′-azobisisobutyronitrile (1 mg) in toluene (1 mL) was added to a stirred solution of **5** (0.1 g, 0.28 mmol) in toluene (2 mL) at 80 °C under argon. The reaction mixture was stirred for 4 h. The solvent was removed, and the residue was flash chromatographed (hexane−EtOAc 80:20) affording 43 mg of **15**. IR, MS, and ^{1}H NMR data of **15** matched those of a tiglate analogue of lychnopholide found in *Eremanthus bicolor*.[18]

Incubations with *Rhizopus oryzae*. *R. oryzae* was first grown in two 500-mL flasks, each containing 100 mL of liquid medium, on a rotatory shaker (200 rpm), at 28 °C, for 48 h. The broth culture obtained, containing the developed biomass in suspension, was inoculated (2 mL per flask) in 500-mL flasks, each containing 100 mL of fresh liquid medium, and a first stage of incubation was carried out in a rotatory shaker (200 rpm), at 28 °C, for 48 h. Then, the exogenous substrates were evenly distributed (30 mg in 0.2 mL of THF per flask) among the first-stage cultures, and a second stage of incubation was carried out at 28 °C on a rotatory shaker. Monitoring of the second-stage cultures was performed by TLC and ^{1}H NMR analysis of aliquot samples taken daily. In all the experiments, two flasks without biomass (control for substrate's chemical stability) and two flasks without exogenous substrate (control for endogenous metabolites) were used.

Biotransformation of Dehydrocostuslactone (4). Lactone **4** (450 mg) was evenly distributed among 15 flasks containing 48-h-old biomass. Biotransformation was stopped after 4 days. Broth culture was filtered, and the filtrate was extracted with EtOAc. The organic solvent was removed, and a crude residue (800 mg) was obtained. Column chromatography of the residue afforded **4** (52 mg, hexane−*t*-BuOMe 90:

10), **16** (293 mg, hexane−*t*-BuOMe 90:10), and **17** (5 mg, hexane−*t*-BuOMe 60:40).

Compound 16: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.20 (1H, d, J = 2.0 Hz, H-15a), 5.03 (1H, d, J = 2.0 Hz, H-15b), 4.89 (1H, br s, H-14a), 4.78 (1H, br s, H-14b), 3.92 (1H, t, J = 10.0 Hz, H-6), 2.88 (1H, dt, J = 8.0, 5.0 Hz, H-1), 2.79 (1H, br dd, J = 10.0, 8.0 Hz, H-5), 2.49 (1H, br dt, J = 12.0, 3.0 Hz, H-9β), 2.21 (1H, dq, J = 11.0, 8.0 Hz, H-11), 1.23 (3H, d, J = 8.0 Hz, H-13); NOE-dif experiments, proton irradiated (NOEs observed) H-15a (H-15b, H-6), H-15b (H-15a), H-14a (H-14b, H-9β), H-14b (H-14a, H-6), H-6 (H-15a, H-14b, H-11); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.5 (s, C-12), 151.8 (s, C-4), 150.0 (s, C-10), 111.9 (t, C-14), 109.3 (t, C-15), 85.4 (d, C-6), 52.0 (d, C-7), 50.0 (d, C-1), 47.2 (d, C-5), 42.2 (d, C-11), 37.7 (t, C-8), 32.6 (t, C-9), 30.3 (t, C-2), 29.1 (t, C-3), 13.3 (q, C-13).

Compound 17: $[\alpha]^{25}_D$ +21.0° (*c* 0.19, $CHCl_3$), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.6 (s, C-4), 108.8 (t, C-15), 84.6 (d, C-6), 58.0 (s, C-10), 50.8 (d, C-7), 50.0 (t, C-14), 49.0 (d, C-1), 45.4 (d, C-5), 42.2 (d, C-11), 36.0 (t, C-8), 32.2 (t, C-9), 28.3 (t, C-2), 24.7 (t, C-3), 13.4 (q, C-13); HRFABMS *m/z* 271.1311 (calcd for $C_{15}H_{20}O_3Na$, 271.1310).

Biotransformation of Lychnopholide (5). Compound **5** (450 mg) was evenly distributed among 15 flasks containing 48-h-old biomass. Biotransformation was stopped after 4 days. Culture filtrate was extracted with EtOAc. Column chromatography of the organic extract (300 mg) yielded **5** (15 mg, hexane−EtOAc 80:20) and **13**[14] (10 mg, hexane−EtOAc 85:15).

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