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Stereoselective first total synthesis, confirmation of the absolute configuration and bioevaluation of botryolide-E

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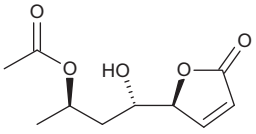
ABSTRACT

A simple, first stereoselective total synthesis of botryolide-E has been described. The synthesis started from propylene oxide employing Jacobsen's hydrolytic kinetic resolution (HKR), selective epoxide opening, sharpless asymmetric dihydroxylation, one pot acetonide deprotection and lactonization as key steps. Further, the synthesis confirms the absolute configuration of the natural product botryolide-E and we evaluated the biological behavior of natural product botryolide-E against a panel of bacteria and fungi. Botryolide-E exhibits significant potent activity against *Staphylococcus aureus* (MTCC 96) (6.25 μg/ml), good against *Escherichia coli* (MTCC 443) (12.5 μg/ml), *Bacillus subtilis* (MTCC 441) (25 μg/ml) and compound **1** exhibited good to moderate antifungal activity.

The emergence of multidrug resistance among pathogens represents a significant challenge for medical professionals.[1] Particular concern are methicillin-resistant *Staphylococcus aureus* (MRSA)[2] and vancomycin-resistant enterococci (VRE), due to the combination of increasing prevalence and recalcitrance to therapy.[3] One important strategy to address these resistance issues is the development of new classes of antibiotic drugs with significant activity against resistant pathogens. In addition, increase in immuno compromised patients and hospital acquired infections has necessitated the exploration of new biological targets and discovery of effective antibacterial and antifungal agents. The natural products containing γ-lactone motif are known to show variety of activities[4] such as cytotoxic,[5] antitumor,[6] cyclooxygenase or phospholipase A2 inhibition.[7] These are of either bacterial or fungal origin. Botryolide-E (**1**), a γ-lactone has been isolated from cultures of the fungicolous *Botryotrichum* sp. (NRRL 38180) by Gloer and co-workers in 2008.[8] The structure and relative configuration was established through a NMR and ESIMS data, respectively. Due to a limitation of isolated compound **1** (1 mg), the authors were unable to evaluate its antibacterial activity.[8] Other isolated compounds such as botryolides A, B, D did not show any activity. In continuation of our interest towards the total synthesis of biologically active lactone containing natural products,[9] we planned to synthesize compound **1** and evaluate the antibacterial activity against *Bacillus subtilis* (MTCC 441), *S. aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 437), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumonia* (MTCC 39) and *Escherichia coli* (MTCC 443) and antifungal activity against *Rhizopus oryzae* (MTCC 262), *Aspergillus niger* (MTCC 1344), *Candida albican* (MTCC 227), and *Saccharomyces cerevisiae* (MTCC 171). To the best our knowledge, the synthesis of **1** has not been reported in the literature and herein we report on simple efficient stereoselective synthesis from commercially available propylene oxide employing Jacobsen's hydrolytic kinetic resolution (HKR), epoxide opening, Sharpless asymmetric dihydroxylation, one pot acetonide deprotection and lactonization as the key steps.



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Retrosynthetically (Scheme 1), we envisaged that our target molecule botryolide-E (**1**) can be obtained from olefinic intermediate **14** by one pot acetonide deprotection and lactonization.

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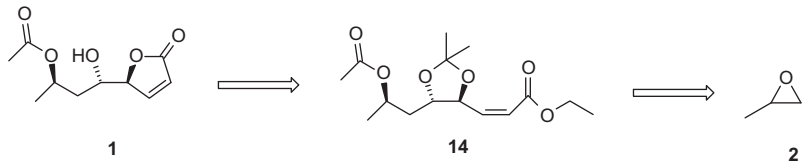


Scheme 1. Retrosynthetic route for botryolide-E.

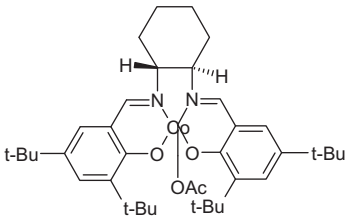
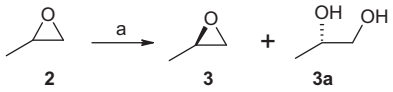


Fig. 1. (*R*,*R*)-salen-Co(III)-(OAc) complex.

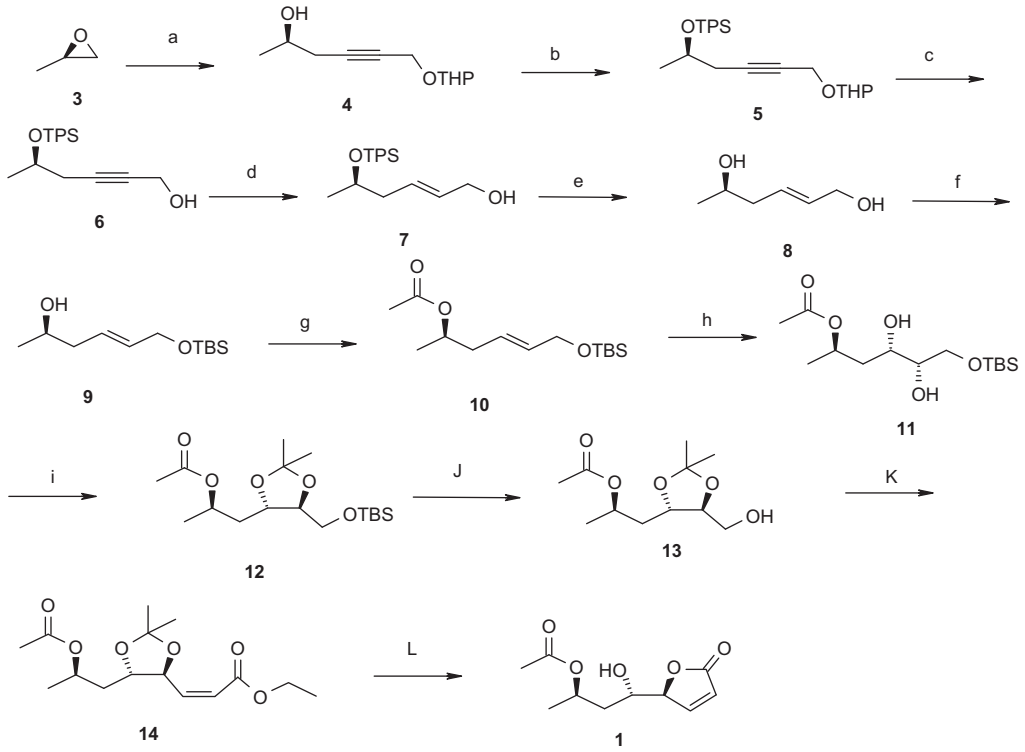


Scheme 2. Reagents and conditions: (a) (*R*,*R*)-salen-Co-(OAc) (0.5 mol%), distd H_2O (0.55 equiv), 0 °C, 14 h, (43% for **3**, 40% for **3a**).

Intermediate **14** in turn prepared from propylene oxide **2** via Jacobsen's hydrolytic kinetic resolution (HKR), selective epoxide opening, sharpless asymmetric dihydroxylation as key steps.

As outlined in Scheme 1, the propylene oxide **2** was subjected to Jacobsen's HKR using (*R*,*R*)-salen-Co-(OAc) catalyst (Fig. 1) to afford *R*-propylene oxide **3** as a single isomer $[\alpha]_D^{25}$ +11.7 (*c* 1, $CHCl_3$), [lit. for *S*-propylene oxide $[\alpha]_D^{25}$ −11.6 (neat)][10] (Scheme 2). The *R*-propylene oxide **3** was easily isolated from the more polar diol **3a** by distillation (Scheme 2).

The enantiomeric pure propylene oxide **3** was subjected to regioselective ring opening with THP protected propargyl alcohol using *n*-BuLi, BF_3OEt to furnish alcohol **4**.[11] The secondary hydroxyl group in **4** was protected with *tert*-butyldiphenylsilyl chloride and imidazole in the presence of a catalytic amount of DMAP to afford the silyl ether **5**, which on depyranylnation using PPTS in methanol afforded compound **6**. The free progargylic alcohol **6** was reduced with Red-Al (3 equiv) to afford allylic alcohol **7**. The *tert*-butyldiphenylsilyl ether in compound **7** was deprotected to form diol **8** in which the primary alcohol was selectively protected using *tert*-butyldimethylsilily chloride to afford monosilyl ether **9**. The secondary hydroxyl group in monosilyl ether **9** was acetylated using acetic anhydride in pyridine to afford acetylated compound **10**. Compound **10** on diastereo and enantioselective Sharpless asymmetric dihydroxylation afforded single diastereomer **11** {$[\alpha]_D^{25}$: −39.5 (*c* 0.25, $CHCl_3$)}.[12] The diol **11** was protected with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-TSA to give compound **12**. The



Scheme 3. Reagents and conditions: (a) $HCCCH_2OTHP$, *n*-BuLi, BF_3OEt, THF, −78 °C, 3 h, 75 %; (b) TBDPSCl, imidazole, cat DMAP, dry CH_2Cl_2, 4 h, 93%; (c) PPTS, MeOH, 0 °C to rt, 5 h, 85%; (d) Red-Al, THF, 0 °C-rt, 12 h, 88%; (e) *p*-TSA, MeOH, 0 °C-rt, 6 h, 93%; (f) TBDMSCl, imidazole, dry CH_2Cl_2, 0 °C-rt, 4 h, 92%; (g) $(Ac)_2O$, Py, 0 °C-rt, 3 h, 93%; (h) ADMIX-α, *t*-BuOH: H_2O (1:1), 0 °C, 24 h, 86 %; (i) 2,2[1]-DMP, dry CH_2Cl_2, PTSA, 1 h, 88%; (j) TBAF, THF, 0 °C-rt, 30 min, 92 %; (k) i. IBX, dry DMSO, dry CH_2Cl_2, 5 h, 92%; ii. PPh_3 = CO_2Et, MeOH, 0 °C, 24 h, 76.1 %; (l) 80% aq AcOH, 0 °C-rt, 24 h, 97%.

Table 1
Antibacterial activity of compound **1**.

Microorganism	Minimum inhibitory concentration (MIC) (μg/ml)			
	Compound **1**	Penicillin-G	Streptomycin	Nitrofurantoin
Gram positive bacteria				
B. Subtilis (MTCC 441)	25	1.562		100
S. aureus (MTCC 96)	6.25	1.562		50
S. epidermides (MTCC 437)	50	1.562		50
Gram negative bacteria				
E. coli (MTCC 443)	12.5		1.562	25
P. aeruginosa (MTCC 741)	50		1.562	100
K. pneumonia (MTCC 39)	50		1.562	50

Minimum inhibitory concentrations (MICs) are in μg/ml. Negative control DMSO – no activity
Note: positive controls: penicillin for Gram positive bacteria.
Streptomycin for Gram negative bacteria.
Nitrofurantoin for both microorganisms.

Table 2
Antifungal activity of compound **1**.

Microorganism	Zone of inhibition (mm)	
	Compound **1**	Claotrimazole
Fungi	100 μg/ml	30 μg/ml
R. oryzae (MTCC 262)	14	21
A. Niger (MTCC 1344)	10	18
C. albicans (MTCC 227)	12	22
S. cerevisiae (MTCC 171)	8	23

Zone of inhibition diameter are in mm. Negative control DMSO – no activity
Note: positive control: claotrimazole for all fungi.

tert-butyldimethylsilyl group in **12** was removed using tetrabutylammonium fluoride (TBAF) to afford alcohol **13**, which was oxidized using IBX (2-iodoxybenzoic acid) and subsequently reacted with (ethoxycarbonylmethylene)triphenylphosphorane in dry methanol at 0 °C for 24 h to give the Wittig product in 82% yield with a *Z:E* ratio of 85:15. The pure *Z* isomer **14** was separated by column chromatography (Scheme 3), which was reacted with 80% aq AcOH at room temperature for 24 h, to afford single product **1** quantitative yield in one pot acetonide deprotection and lactonization (Scheme 3). The physical and spectral data of synthetically prepared compound [15] **1** (^{1}H NMR and ^{13}C NMR) were found to be in good agreement the natural product[8] {$[\alpha]_D^{25}$ −36.7 (*c* 0.05, $CHCl_3$), lit.[8] $[\alpha]_D^{25}$ −38 (*c* 0.05, $CHCl_3$)} there by confirming the absolute configuration of natural product botryolide-E 1 as 4*S*, 5*S* and 7*R* configuration.

The in vitro biological activity of botryolide-E against bacteria was evaluated. All the microbial culture were procured from MTCC, IMTECH, Chandigarh, India. The antibacterial activity of botryolide-E **1** was tested against a panel of bacteria by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards procedure,[13] and minimum inhibitory concentrations (MICs) are summarized in Table 1. It has been observed that the test compound botryolide-E exhibited good antibacterial activity with degree of variation. Natural product botryolide-E (**1**) exhibited significant potent activity against *S. aureus* (MTCC 96) (6.25 μg/ml), good activity against *E. coli* (MTCC 443) (12.5 μg/ml) and *B. subtilis* (MTCC 441) (25 μg/ml) and exhibits moderate activity against *S. epidermidis* (MTCC 437) (50 μg/ml) and *P. aeruginosa* (MTCC 741) (50 μg/ml),*K. pneumonia* (MTCC 39) (50 μg/ml). Controls were maintained with DMSO and penicillin-G (for Gram positive bacteria), streptomycin (for Gram negative bacteria) and nitrofurantoin for all bacteria were used as positive controls, respectively. MIC values of standard drugs penicillin-G, streptomycin and nitrofurantoin against bacteria are provided in Table 1. Agar cup bioassay method was employed for testing antifungal activity of compound **1** following the standard procedure[14] and zone of inhibitions (ZOIs) are summarized in Table 2. The antifungal activity of compound **1** was studied against *R. oryzae* (MTCC 262), *A. niger* (MTCC 1344), *C. albican* (MTCC 227), and *S. cerevisiae* (MTCC 171). Compound **1** showed good activity with zone of inhibition values in the range of 12–14 mm at a compound concentration of 100 μg/ml against *R. oryzae* (MTCC 262) and *C. albican* (MTCC 227) and showed moderate activity with zone of inhibition values in the range of 8–10 mm at a compound concentration of 100 μg/ml against *A. niger* (MTCC 1344) and *S. cerevisiae* (MTCC 171). The controls were maintained with clotrimazole for all fungi. Zone of inhibition values for standard drug clotrimazole for all fungi are presented in Table 2.

In conclusion, the synthesis of botryolide-E from propylene oxide has been achieved employing Jacobsen's hydrolytic kinetic resolution (HKR), selective epoxide opening, Sharpless asymmetric dihydroxylation, one pot acetonide deprotection and lactonization as key steps, there by confirming the absolute configuration as 4*S*, 5*S* and 7*R*. Biological behavior of botryolide-E against bacteria and fungi has been evaluated. Botryolide-E shows significant potent activity against *S. aureus* (MTCC 96) (6.25 μg/ml), good against *E. coli* (MTCC 443) (12.5 μg/ml), *B. subtilis* (MTCC 441) (25 μg/ml) and moderate activity against *S. epidermidis* (MTCC 437) (50 μg/ml), *P. aeruginosa* (MTCC 741) (50 μg/ml) and *Klebsiella pneumonia* (MTCC 39) (50 μg/ml). Compound **1** exhibited good to moderate antifungal activity.

Experimental section. General experimental procedures are described in the Supplementary data.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.024.

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15. *Spectral data for selected compounds:* Compound **11**: $[\alpha]_D^{25}$: −39.5 (*c* 0.25, $CHCl_3$). IR (Neat): υ 3435, 2925, 1716, 1643, 1378, 1267 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.12 (ddq, *J* = 12.1, 6.8, 4.2 Hz, 1H), 3.67 (dd, *J* = 5.0, 2.4 Hz, 2H), 3.64–3.60 (m, 1H), 3.42–3.24 (m, 1H) 3.07 (br s, OH), 2.63 (br s, OH), 2.04 (s, 3H), 1.76 (ddd, *J* = 14.1, 11.1, 3.0 Hz, 1H), 1.62 (ddd, *J* = 14.3, 10.0, 2.8 Hz, 1H), 1.28 (d, *J* = 6.2 Hz, 3H), 0.9 (s, 9H), 0.08 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 67.8, 67.6, 66.9, 39.9, 29.9, 29.5, 25.6, 21.2, 19.4, −2.9, −3.6. ESIMS: *m*/*z* 329 $[M + Na]^+$.
Compound **1**: $[\alpha]_D^{25}$: −36.7 (*c* 0.05, $CHCl_3$). IR (Neat): υ 3424, 2924, 2854, 1725, 1739, 1663, 1250.cm^{-1}.^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (dd, 1H, *J* = 6.0, 1.5 Hz), 6.20 (dd, 1H, *J* = 6 0, 1.5 Hz), 5.13–5.08 (m, 1H), 5.05–5.01 (m, 1H), 3.88–3.85 (m, 1H), 3.23 (br s, OH), 2.04 (S, 3H), 1.72–1.81 (m, 2H), 1.27 (d, 3H, *J* = 6.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 172.0, 153.4, 123.0, 85.0, 67.6, 67.3, 39.1, 21.2, 20.6. ESIMS: *m*/*z* 214 $[M]^+$.