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Eremophilane Sesquiterpenes from Capsidiol

Yuxin Zhao,[†] David J. Schenk,[†,§] Shunji Takahashi,[‡] Joe Chappell,[‡] and Robert M. Coates*[,†]

Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801, and Department of Agronomy, Plant Physiology, Biochemistry, and Molecular Biology Program, University of Kentucky, Lexington, Kentucky 40546-0091

coates@scs.uiuc.edu

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A series of eremophilane sesquiterpene alcohols and hydrocarbons was prepared from the phytoalexin capsidiol (**1**) for mechanistic studies with epiaristolochene synthase and epiaristolochene dihydroxylase. Among them, 3-deoxycapsidiol (**10**) was obtained through selective derivatization and reductive cleavage of the equatorial 3α hydroxyl group. Two novel isomers of aristolochene and eremophilene were accessed from the 1- and 3-deoxycapsidiol isomers. 4-Epieremophilene (**17**) was obtained by conjugate reduction of epiaristolochen-1-one tosylhydrazone with catecholborane followed by sulfinate elimination and diimide rearrangement. Epimerization of epiaristolochen-3-one (**27a**) at the C4 methyl followed by reductions led to the previously unknown aristolochene isomer, eremophila-9(10),11(12)-diene (**30**). Optical rotations and characteristic ^{1}H NMR data for the related eremophilenols and dienes are collected in Tables 1 and 2. Finally, bioassays were used to assess the antifungal potencies of capsidiol and its synthetic derivatives. The minimum inhibitory concentration for capsidiol (3−10 ng) was at least 1 order of magnitude lower than that of any of the derivatives and considerably lower than those previously reported for ketoconazole, nystatin, and propiconazole.

Introduction

Capsidiol (**1**)[1] is an eremophilane-type sesquiterpene[2] that occurs in many solanaceous species. This natural product exhibits antifungal activity[3] and is considered to be the primary phytoalexin[4] biosynthesized by tobacco and pepper plants in response to various environmental stimuli.[5] The structure first proposed on the basis of chemical transformations and NMR spectral evidence[6a] was verified by X-ray diffraction analysis,[6b] and the absolute configuration was assigned by the exciton chirality method with capsidiol dibenzoate.[7]

The biosynthetic precursor, 5-epiaristolochene (**2**),[8] was first obtained as the major product from incubation of (*E*,*E*)-farnesyl diphosphate (FPP, **6**) with a cell-free enzyme extract from elicitor-treated suspension cultures of *Nicotiana tabacum*.[9] The structure of epiaristolochene was confirmed by independent synthesis of the sesquiterpene by reductive deoxygenations of capsidiol.[10] The stereochemistry of the vicinal methyl groups and isopropenyl substituent differs from those of the related eremophilane sesquiterpene (−)-aristolochene (**3**) that occurs in *Aristolochia indica* and *Bixa orellana*,[11a] valencene (**4**) from orange peel oil,[11b] and the venerable eremophilone (**5**) from rhizomes of *Petasites officinalis*[12] (Figure 1), the first terpene to violate the structural isoprene rule. The enantiomer of **3**, (+)-aristolochene isolated from *Aspergillus terreus*,[13] is regarded as the likely precursor

* Author to whom correspondence should be addressed.
† University of Illinois.
‡ University of Kentucky.
§ Present address: Department of Drug Metabolism, Merck Research Laboratories, RY80R-104, P.O. Box 2000, Rahway, NJ 07065.

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(8) Preferred semisystematic names for capsidiol (**1**) and 5-epiaristolochene (**2**) should be based on the eremophilane parent (i.e., 4-epieremophila-9(10),11(12)-diene-1*β*,3α-diol and 4-epieremophila-9(10),11(12)-diene, respectively). In this paper, compounds having the ring double bond in the 9,10 position are designated as derivatives of either capsidiol (e.g., 1-deoxycapsidiol, **9**) or 5-epiaristolochene (e.g., epiaristolochen-3α-ol, **9**) as seems to be appropriate for the context since these names are commonly used in the literature. On the other hand, **12**, having the ring double bond in the 1,10 position as it is in eremophilone (**5**), is designated as 4-epieremophila-1(10),11(12)-dien-3α-ol or simply 4-epieremophilen-3α-ol. In certain cases, names based on both epiaristolochene and eremophilane are shown for clarity.

(9) Whitehead, I. M.; Threlfall, D. R.; Ewing, D. F. *Phytochemistry* **1989**, *28*, 775−779.

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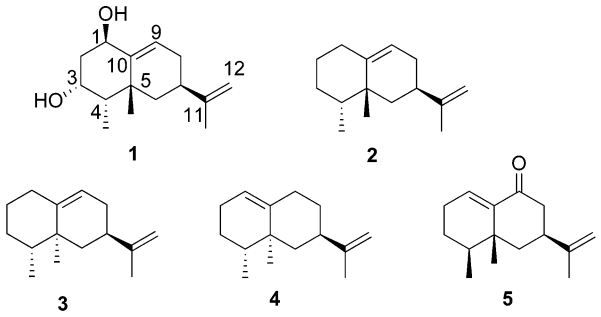
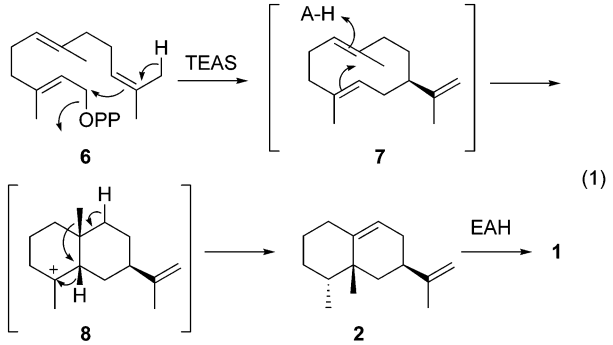


FIGURE 1. Structures of capsidiol and related eremophilane sesquiterpenes.

of sesquiterpene toxins elaborated by other fungal species. Isotope labeling experiments confirmed the occurrence of the proposed syn hydride and methyl shifts in capsidiol biosynthesis.[14]

The elicitor-induced genes associated with the biosynthesis of this sesquiterpene phytoalexin and the corresponding enzymes and their catalytic mechanisms have recently been the subjects of considerable research. Epiaristolochene synthase from *N. tabacum* (TEAS) was cloned and heterologously expressed in *Escherichia coli*,[15] and the crystal structure of the novel cyclase was elucidated by Chappell, Noel, and co-workers.[16] The intermediacy of germacrene A (**7**) in the cyclization mechanism shown in eq 1 was supported by the isolation of the macrocyclic triene from incubation of FPP with the TEAS mutant Tyr520Phe, indicating a possible role of the phenolic OH in the further cyclization of the intermediate.[17] Functional domains of TEAS were identified by domain swapping with the premnaspiradiene synthase gene from *Hyoscyamus muticus*.[18] Pre-steady-state kinetics of recombinant TEAS catalysis revealed the slow conversion of FPP to a hydrocarbon intermediate followed by a rate-limiting step, probably the release of the hydrocarbon product.[19] Biochemical evidence with tobacco[9] and peppers[20] indicated that oxidation of epiaristolochene was effected by at least one elicitor-inducible cytochrome P450 hydroxylase. A single inducible P450 enzyme, CYP71D20, expressed in yeast was capable of converting both epiaristolochene and 1-deoxycapsidiol to capsidiol in vitro.[21]



Since capsidiol is readily available by cellulase elicitation of green peppers,[20] we decided to use the natural product as the starting material for preparing a number of eremophilane sesquiterpene alcohols and hydrocarbons for ongoing investigations on the mechanism and specificity of epiaristolochene synthase and epiaristolochene dihydroxylase.[21,22] In this paper, we report the synthesis and characterization of 3-deoxycapsidiol (**10**), a possible intermediate in the biosynthesis of capsidiol. Also recorded are syntheses of novel isomers of aristolochene and eremophilene together with comparative antifungal activity of the various capsidiol analogues.

Biosynthesis of Capsidiol

It is well established that exposure of tissue cultures of whole peppers or tobacco plants to fungal spores leads to enhanced levels of capsidiol, in accord with its function as a phytoalexin.[1,5] We followed the convenient laboratory procedure reported by Whitehead et al.[20] in which whole bell pepper fruits are inoculated with aqueous cellulase and capsidiol is isolated by extraction of the aqueous suspension after 24 h. We found that the yield was approximately doubled to ~3 mg/pepper by extending the incubation time to 3 days and extracting with CH_2Cl_2 instead of ether.

Deoxycapsidiols. Samples of 1- and 3-deoxycapsidiols (5-epiaristolochen-3α- and -1β-ols, **9** and **10**, respectively) were required for an investigation on the kinetics of EAH oxidations and to determine the sequence of the hydroxylation steps (eq 2).[22c,d] The known reductive cleavage of capsidiol diacetate with Li/NH_3[10] was repeated, and the resulting 1-deoxycapsidiol and its regioisomer, 4-epieremophilen-3α-ol (**9** and **12**, 8−12:1, 78%), were separated by flash chromatography (eq 3).

Although 3-deoxycapsidiol (**10**) is mentioned in the literature,[23] the source or method of synthesis was not given and no physical data were provided. Since a previous report indicated that capsidiol could be selectively converted to its 3-mesylate,[6a] we expected that

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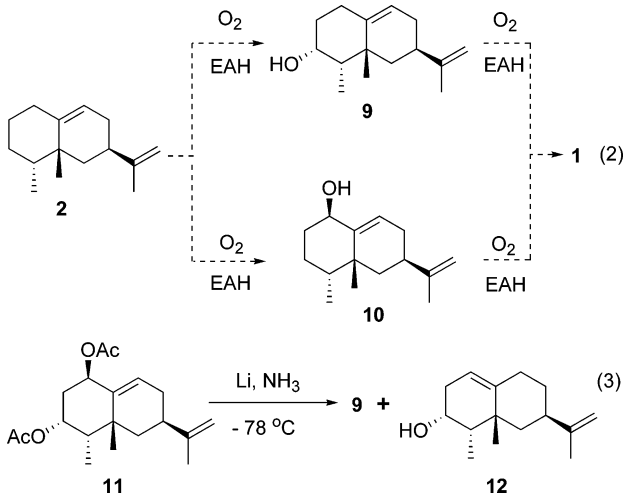
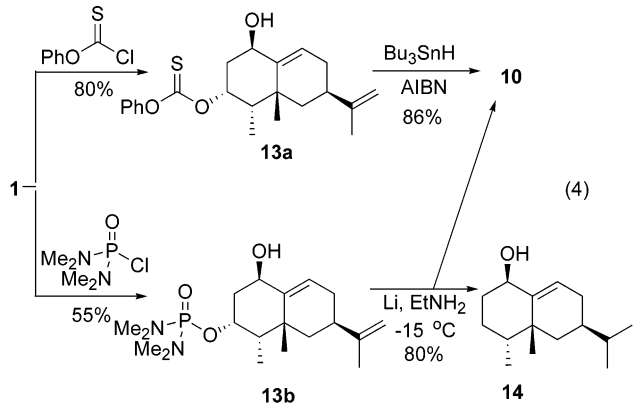
(22) (a) Schenk, D. J., Stereochemistry and Mechanism of the Enzyme-Catalyzed Cyclizations of Farnesyl Diphosphate to the Sesquiterpenes Epiaristolochene, Epieremophilene, and Vetispiradiene, Ph.D. Dissertation, University of Illinois, Urbana, IL, 2000. (b) Greenhagen, B. T., Origins of Isoprenoid Diversity: A Study of Structure-Function Relationships in Sesquiterpene Synthases, Ph.D. Dissertation, University of Kentucky, Lexington, KY, 2003. (c) Takahashi, S.; Greenhagen, B. T.; Zhao, Y.; Coates, R. M.; Chappell, J. Kinetic Mechanism for Successive Hydroxylation by 5-Epi-aristolochene-1,3-dihydroxylase. Presented at the 6th International Symposium on Isoprenoids and Terpenes, May 14−17, 2003, Lexington, KY. (d) Takahashi, S.; Zhao, Y.; O'Maille, P. E.; Greenhagen, B. T.; Noel, J. P.; Coates, R. M.; Chappell, J., manuscript in preparation.

(23) Hoshino, T.; Yamaura, T.; Imaishi, H.; Chida, M.; Yoshizawa, Y.; Higashi, K.; Ohkawa, H.; Mizutani, J. *Phytochemistry* **1995**, *38*, 609−613.



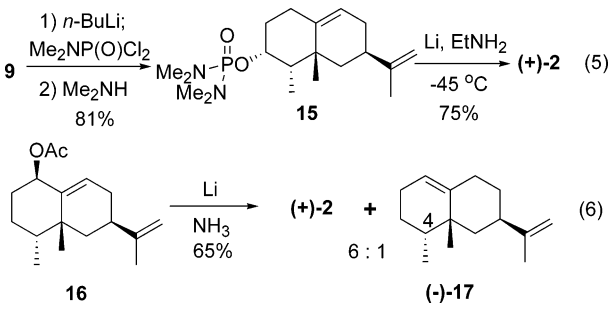
regioselective derivatizations of the less-hindered, equatorial hydroxyl group would be possible (eq 4). The reaction of capsidiol with phenyl chlorothionoformate (1.3 equiv, pyr-CH_2Cl_2, room temperature, 8 h)[24] afforded 3-phenyl thionocarbonate **13a** (80%). The position of the installed thiocarboxyl group was evident from the downfield shift and multiplicity of the axial proton at C3 (6.10, dt, J = 12.3 and 4.5 Hz). Reductive cleavage of the thionocarbonate with Bu_3SnH generated in situ [$(Bu_3Sn)_2O$, AIBN, polymethylhydrosiloxane, PhH, reflux, 5 h][24] afforded 3-deoxycapsidiol (**10**) in 86% yield. The 3α hydroxyl group of capsidiol was also removed by regioselective conversion to the crystalline monophosphoramidate **13b** (55%) and subsequent reduction with Li/$EtNH_2$ at −15 °C.[25] The components of the resulting 5:1 mixture of 3-deoxycapsidiol and the corresponding 11,12-dihydro byproduct (**14**), arising from further reduction of the isopropenyl double bond, were separated by preparative HPLC.



Selected physical properties of the deoxycapsidiol isomers and 4-epieremophilen-3α-ol, together with those of capsidiol, are presented in Table 1. The greater accessibility of the equatorial OH group at C3 in **9** and **12** is presumably responsible for the increased relative polarity of these compounds in comparison to that of 3-deoxycapsidiol (**10**). The lower field positions of the ring vinyl protons and angular methyl groups in the ^{1}H NMR spectra of **1** and **10** are attributed to the inductive and shielding influences of the axial, allylic OH at C1. The NMR data for 4-epieremophilen-3α-ol (**12**) are quite similar to those of its double-bond isomer, epiaristolochen-3α-ol (**9**).[10] However, the chemical shift of the endocyclic vinyl H in **12** is almost 0.4 ppm upfield compared to those of the three compounds with double bonds in the 9,10 position. Furthermore, the coupling constants for the ring proton at C3 (3.5, 5.8, and 9.8 Hz) indicate that the conformation of the cyclohexene ring is inverted compared to those of compounds **1**, **9**, and **10** (conformational depictions for the parent hydrocarbons **2** and **17** are presented in Figure 2). The interpretations of the NMR data for **12** are consistent with the literature.[10]

A conspicuous one-proton multiplet, visible in most NMR spectra of the functionalized sesquiterpenes due to its relatively high field position (δ_H 1.40−1.05, not shown in Table 1), could be assigned to Hβ on the C6 methylene position. In 16 such compounds having Δ9-(10) ring double bonds, H6β appears as a triplet or closely spaced doublet of doublets with large, nearly equal J values (∼12−13 Hz) arising from coupling to its geminal and antiperiplanar neighbors. However, in two of the three compounds having the Δ1(10) ring double bond, H6β is found at somewhat lower field (δ_H 1.40 and 1.39) as a more pronounced doublet of doublets (J = 13.8 and 9.7−9.8 Hz). The diminished vicinal coupling interaction is presumably caused by some rotation about the C6−C7 bond that relieves the syn 1,3 diaxial interaction between the angular methyl and isopropenyl groups.

4-Epiaristolochene, 4-Epieremophilene, and 4,7-Diepiaristolochene. Samples of epiaristolochene and 4-epieremophilene (**2** and **17**, respectively) were needed for identification of the sesquiterpene products formed in incubations with TEAS and its mutant forms[22] and for planned experiments with EAH.[22c] The Δ9 isomer was obtained both by Li/$EtNH_2$ reduction of the phosphoramidate **15** at −45 °C[26] (eq 5, 75%) and by Li/NH_3 reduction of 3-deoxycapsidiol acetate (eq 6, 53%).[10] The lower temperature (−45 vs − 15 °C) of the Li/$EtNH_2$ conditions avoided overreduction in this case (eq 5 vs eq 4). Unfortunately, the components of the 6:1 mixture of **2** and **17** formed in the Li/NH_3 reduction (eq 6) were not readily separated by chromatography.



The predominance of the Δ9(10) double bond isomers in the Li/NH_3 reductions of allylic acetates **11** and **16** (eqs 3 and 6)[10] can be understood by considering the confor-

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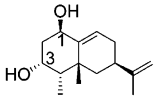
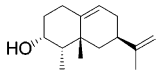
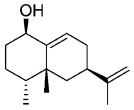
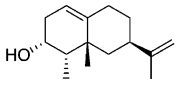
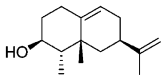
(26) Das, J.; Valenta, Z.; Liu, H.-J.; Ngooi, T. K. *Can. J. Chem.* **1984**, *62*, 481−483.

TABLE 1. Structures, Numbers, Optical Rotations, and Selected ^{1}H NMR Data for Capsidiol and Deoxycapsidiol Isomers

Structure	No.	$[\alpha]^{25}_D$ [a]	TLC R_f [b]	^{1}H NMR [c] [δ_H, m, *J* value(s)]			
				C*H*OH	C=CH	CHCH_3	CCH_3
	**1**	+21 [d]	0.05	4.36 dd, 2.3, 3.7 (H1) 4.59 dt 4.5, 12.5 (H3)	5.93 dd, 2.0, 7.0	0.88 d, 7.0	1.37 s
	**9**	+27.3 [e]	0.27	4.21 dt, 4.7, 12.0	5.64 dt, 1.5, 7.0	0.92 d, 7.0	1.16 s
	**10**	-12.7	0.36	4.25 t, 2.7	5.87 dd, 2.1, 6.8	0.95 d, 7.0	1.36 s
	**12**	-140 [f]	0.28	4.18 ddd, 3.5, 5.8, 9.8	5.22 dt, 2.5, 4.5	0.94 d, 7.0	1.18 s
	**31**	+ 0.25 [g]	0.34	3.83 q, 3.0	5.58 dt, 2.0, 6.5	0.95 d, 7.5	1.39 s

[a] $CHCl_3$. [b] Solvent system of 4:1 hexane/ethyl acetate. [c] 400 or 500 MHz, $CDCl_3$. [d] The same value is reported in ref 6a. [e] From ref 10 (*c* = 0.6, $CHCl_3$). [f] Error range: ±10. [g] Error range: ±0.05.

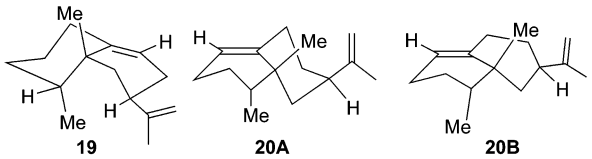
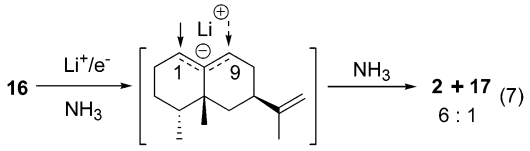
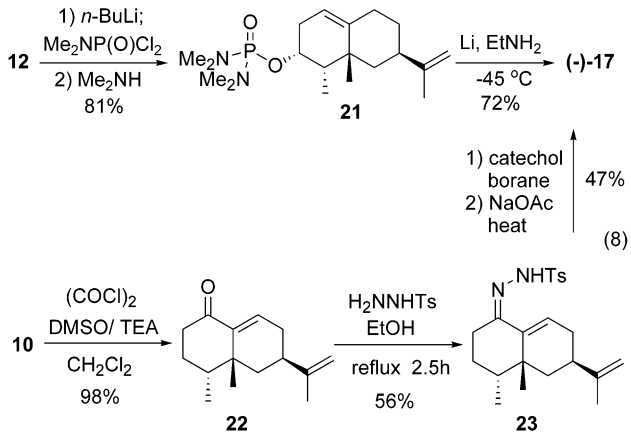


FIGURE 2. Conformational depictions of epiaristolochene (**2** = **19**) and epieremophilene (**17** = **20A** and **20B**).

mations of the regioisomeric diene products (Figure 2). The reactions presumably generate transient allylic carbanions (eq 7, **18**) that undergo rapid irreversible protonation through a product-like transition state to form predominantly the thermodynamically more-stable isomer (eq 7).[27] In the chair−half-chair conformer **19**, with the 9,10 double bond, the isopropenyl substituent is situated in the less-crowded equatorial position. However, with the double bond in the 1,10 position, the isopropenyl group is constrained to an axial position in the half-chair−chair conformer **20A**. The resulting steric interaction with the angular methyl group may be relieved by a ring flip to the half-chair−boat conformation **20B**, albeit, with an increase in torsional strain. This conformational analysis leads to the prediction that isomers having the double bond in the 9,10 position should be somewhat more stable.



4-Epieremophilene (**17**) was first obtained in pure form by Li/$EtNH_2$ reduction of phosphoramidate **21** (eq 8)[22a] that was prepared from small amounts of 4-epieremophilen-3α-ol (**12**) accumulated from the Li/NH_3 reductions of capsidiol diacetate (eq 3).[10] Larger quantities of pure (−)-**17** were obtained by conjugate reduction of epiaristolochen-1-one tosylhydrazone (**23**) with catecholborane followed by NaOAc-induced α elimination, as illustrated in the bottom half of eq 8.[28] The major product was isolated by chromatography on $AgNO_3$-silica gel to separate the 11,12-dihydro byproduct (structure not shown; GC-MS *m*/*z* 206), presumably formed by diimide reduction of the isopropenyl double bond. No significant amount of epiaristolochene (**2**) was seen in the GC of the product mixture.

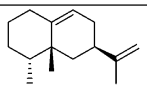
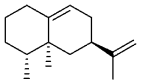
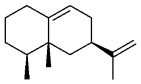
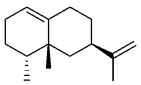
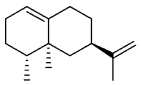
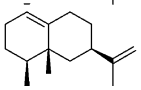


The racemic form of 4-epieremophilene (±)-**17** was prepared from trans,cis ester (±)-**24**, available from the previous work completed in this laboratory on the total

(27) The tendency of Li/NH_3 reductions to form the more stable product is well established; see: Caine, D. *Org. React.* **1976**, *23*, 1.

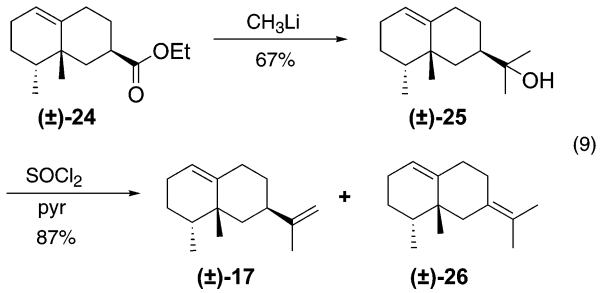
(28) (a) Kabalka, G. W.; Hutchins, R.; Natale, N. R.; Yang, D. T. C.; Broach, V. *Organic Syntheses*; Wiley: New York, 1988; Collect. Vol. 6, pp 293−298. (b) Chu, M.; Coates, R. M. *J. Org. Chem.* **1992**, *57*, 4590−4597.

TABLE 2. Structures, Numbers, Optical Rotations, and Selected ^{1}H NMR Data for Aristolochene and Eremophilene Stereoisomers

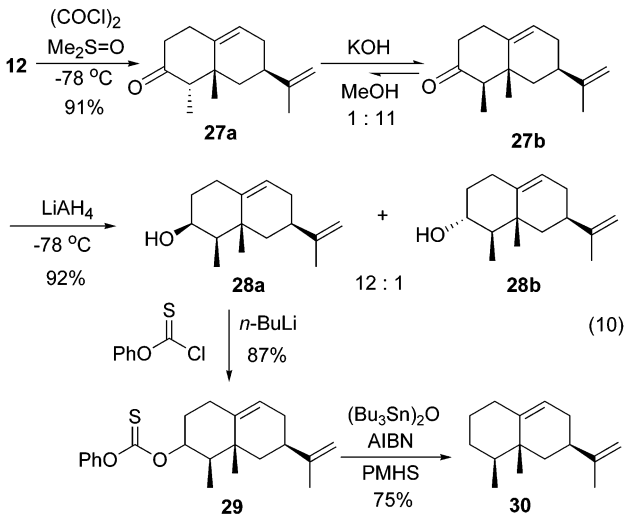
Structure	No.	$[\alpha]^{25}_D$	^{1}H NMR[a] [δ_H, m, J value(s)]		
			C=CH	CHCH_3	CCH_3
	**2**	+ 8.13[b]	5.53 dt, 1.9, 6.0	0.98 d, 7.2	1.17 s
	**3**	- 75.6[c]	5.25[c]	0.83 d, 6.0[c]	0.95 s[c]
	**30**	- 11.8	5.35 tt, 1.8, 6.3	0.80 d, 6.6	0.93 s
	**17**	- 22.7	5.40 td, 1.5, 3.7	0.92 d, 7.0	1.15 s
	**4**	+ 73.4[d]	5.33 m[d]	0.87 d, 6.4[d]	0.95 s[d]
	**32**	- 142.5[e]	5.31 m[e]	0.86 d[e]	0.91 s[e]

[a] 500 MHz, $CDCl_3$. [b] From ref 10 (c = 0.16, hexane). [c] Optical rotation (c = 0.078, hexane) and ^{1}H NMR data from ref 30. [d] Optical rotation from ref 31a (c = 0.53, $CHCl_3$); ^{1}H NMR data from ref 31b. [e] From ref 32.

synthesis of eremophilane sesquiterpenes (eq 9).[29] Conversion of **24** to 4-epieremoligenol (±)-**25** was accomplished by the addition of methyllithium (ether, 0 °C, 1 h, 67%). Dehydration with thionyl chloride ($SOCl_2$, pyr, 0 °C, 20 min) afforded (±)-4-epieremophilene and its tetrasubstituted double-bond isomer (±)-**26** in 43 and 15% isolated yields, respectively. The regioisomers were separated by chromatography on $AgNO_3$-silica gel (50:1 pentane:ether). This work comprises the first syntheses of 4-epieremoligenol and 4-epieremophilene.



The presence of the C4 chiral center adjacent to the C3 hydroxyl group in 1-deoxycapsidiol opened the way to isomerization by equilibration of the axial C4−CH_3 to the equatorial position, as shown in eq 10. Thus, Swern oxidation of **12** gave epiaristolochen-3-one (**27a**, 91%). Exposure of the ketone to KOH/MeOH effected predominant epimerization to give the all-cis isomer **27b** (cis/trans = 92:8). $LiAlH_4$ reduction predominantly gave the all-cis dienol **28a** (^{1}H NMR: equatorial C*H*OH, 3.92, q, J = 3.0 Hz) as a consequence of hydride attack opposite to the angular methyl group (12:1, 92%). Conversion to thionocarbonate **29** (PhOC(=S)Cl, pyr/CH_2Cl_2, 87%) followed by free radical reduction with in situ-generated Bu_3SnH ($(Bu_3Sn)_2O$, AIBN, PMHS)[25] afforded the previously unknown 4,5-diepiaristolochene (eremophila-9(10),-11(12)-diene, **30**). Reduction of enone **27a** ($LiAlH_4$, ether, −78 °C) afforded a 1:11 mixture (92%) of 1-deoxycapsidiol (**9**) and its 3β-ol isomer (**31**; see Table 1).



Optical rotations and selected ^{1}H NMR data for the eremophilene-type sesquiterpenes are presented in Table 2. Although the NMR data for isopropenyl groups in these compounds do not vary appreciably, some consistent differences were observed in comparisons of endocyclic vinyl H and two methyl signals among the isomers. Thus, a relatively large coupling constant for the ring vinyl H in isomers **2** (6.0 Hz) and **30** (6.3 Hz) contrasts with the reduced coupling when the double bond is located in the 1,10 position (**17**, **4**, and **32**). This difference can be explained by the different conformations of the cyclohexene rings with the double bond in the 1,10 or 9,10 positions (see eq 7). When the double bond is located in the 9,10 position, the dihedral angles of the vinyl protons and two adjacent allylic protons are small. However, if it is in the other ring, the steric interaction between the cis methyl and isopropenyl group presumably forces the ring into a half-boat conformation; thus, one of the allylic

(29) Coates, R. M.; Shaw, J. E. *J. Org. Chem.* **1970**, *35*, 2597−2610.

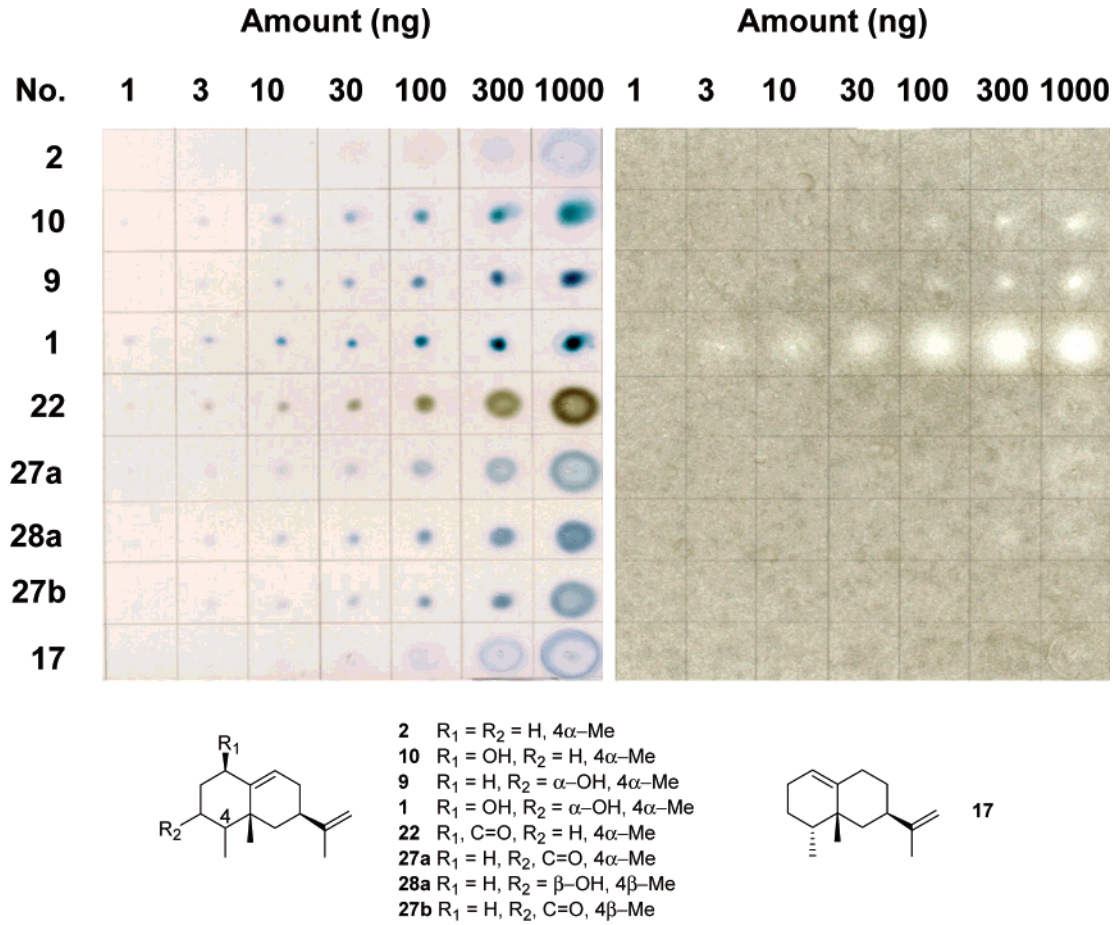


FIGURE 3. Antifungal activity assay of capsidiol and its derivatives.

protons will be eclipsed to the vinyl H, thus reducing the coupling to around 6 Hz. The characteristic high-field triplet from the H6β proton (δ_H 1.28, 1.17, and 1.12) can also be observed in the spectra of the three Δ(9,10) isomers prepared in this work (**2**, **3**, and **30**).

When the methyl group on C5 is cis to the methyl group on C4, as in **3**, **4**, and **30**, the chemical shifts are upfield by 0.2 ppm compared to those of the C5 methyl signals in compounds **2** and **17**. Furthermore, the C4 methyl signals in the isomers with cis methyl relationships are also upfield compared to those of their trans counterparts. The relatively high field position of the cis methyl groups seems to be logically attributed to steric crowding and the cumulative anistropic influence of the proximal C−C bonds.

Antifungal Activities. Since capsidiol has been characterized as a key defense compound produced by several solanaceous plants in response to microbial attack,[4,5] the efficacy of the capsidiol analogues for inhibiting fungal spore germination and hyphal growth was also investigated. Aliquots of solutions of eight purified compounds (**2**, **9**, **10**, **17**, **27a**, **27b**, **28a**, and **22**) along with capsidiol (**1**) were arrayed on silica TLC plates (Figure 3) and either visualized with a vanillin−H_2SO_4 developing reagent (left panel, Figure 3) or subjected to a fungal overlay bioassay (right panel, Figure 3). Antifungal activity is evident as white zones, reflecting an inhibition of spore germination and hyphal growth. Somewhat surprising was the observation that only capsidiol and the two 1- and 3-deoxycapsidiols (**9** and **10**, respectively) exhibited significant antifungal activity, while no such activity was evident either with the eremophilene hydrocarbons (**2** and **17**) or with the monoketones (**27a** and **27b**). The minimum inhibitor concentration (MIC) observed for capsidiol (**1**) was 3 ng/spot, which is 10-fold below that required for compounds **9** and **10** (30 ng/spot), indicating the importance of hydroxylation at both the C1 and C3 positions for full antifungal activity. However, the lack of antifungal activity with compound **28a** suggests that hydroxylation at position C1 or C3 is not sufficient for activity, and that the stereopositioning of the methyl substituent at C15 does influence biological activity. Equally interesting is that the MIC value for capsidiol (3−10 ng/spot) is much lower than those reported for the known antifungal agents ketoconazole (100 ng/spot),[35] nystatin (1250 ng/spot),[35] miconazole (1000 ng/spot),[35,37] and propiconazole (10−100 ng/spot).[36,38]

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Conclusion

Epieremophilane sesquiterpenes are readily accessible by functional modifications of capsidiol. Both deoxycapsidiol isomers **9** and **10** are now available for kinetic studies to elucidate the sequence of oxidation steps effected by epiaristolochene dihydroxylase. The novel epimers and regioisomers of epiaristolochene characterized in this work have an important role in studies on the specificity and mechanism of epiaristolochene synthase, dihydroxylase, and their mutant forms.[22]

Experimental Section

Elicitation and Isolation of Capsidiol (1) from Green Peppers.[20,22a,39] Two opposing needle holes were made in the upper portion of 150 green bell pepper fruits with a 16-gauge needle. The peppers were filled with a 1.1 mg/L solution of cellulase (*Tricoderma viride*) in deionized water by a gravity feed through a 0.25-in. tube ending with a short Pasteur pipet. After 72 h at room temperature, the sides of the peppers were cut open and the aqueous solution was filtered through cheesecloth into 20-gallon plastic barrels. Batches (1 L) of the combined aqueous solution were extracted with CH_2Cl_2 (2 × 0.25 L). Concentration of the combined CH_2Cl_2 layers and purification by flash chromatography (1:6 hexane/ethyl acetate) provided 0.452 g (3.0 mg/pepper) of white crystalline capsidiol. The purity was shown to be essentially 100% by GC and ^{1}H NMR analyses: mp (uncorrected) 149.5−151 °C (lit.[6a] mp 152−153 °C); TLC R_f = 0.21 (1:6 hexane:ethyl acetate); $[\alpha]^{25}_D$ = +21.3 (c = 1.75, $CHCl_3$) [lit.[6a] $[\alpha]^{25}_D$ = +21 (c = 2.1, $CHCl_3$)]; GC (100%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.93 (dd, 1H, J = 7.0, 2.0 Hz), 4.72 (t, 1H, J = 1.5 Hz), 4.69 (d, 1H, J = 1.0 Hz), 4.59 (dt, 1H, J = 12.5, 4.5 Hz), 4.36 (dd, 1H, J = 3.7, 2.3 Hz), 2.18 (tt, 1H, J = 12.3, 3.5 Hz), 2.08 (dddd, 1H, J = 16.5, 6.7, 4.0, 3.0 Hz), 1.94 (d of septets, 1H, J = 13.5, 1.0 Hz), 1.87 (ddd, 1H, J = 16.5, 11.5, 2.0 Hz), 1.80 (dt, 1H, J = 14.0, 3.0 Hz), 1.75 (m, 1H), 1.74 (s, 3H), 1.66 (ddd, 1H, J = 13.5, 12.5, 4.0 Hz), 1.37 (m, 2H), 1.37 (s, 3H), 1.33 (dd, 1H, J = 13.9, 13.1 Hz), 0.88 (d, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.4, 140.3, 129.0, 108.7, 75.0, 65.4, 47.6, 44.9, 40.1, 39.1, 36.1, 32.1, 30.3, 21.0, 8.9. The NMR data agree with the literature values.[10] However, more complete analyses and assignments are given above.

Capsidiol 3-Phenylthionocarbonate (13a). The general procedure of Fu[24] was followed. A solution of capsidiol (**1**) (85 mg, 0.36 mmol, 1.0 equiv) in pyridine (1.4 g, 1.5 mL, 18 mmol) and CH_2Cl_2 (1.5 mL) was stirred and cooled at 0 °C as phenyl chlorothionoformate (65 μL, 0.47 mmol, 1.3 equiv) was added over 2−3 min. After 10 min, the solution was warmed to room temperature and stirred for 8 h. MeOH (25 μL, 0.61 mmol) was added, and the reaction mixture was stirred for another 1 h. The solution was diluted with Et_2O (15 mL), and aqueous HCl (1 M, 10 mL). The aqueous layer was extracted with Et_2O (3 × 25 mL). The combined Et_2O extracts were washed with saturated $Cu(NO_3)_2$ (2 × 15 mL) and H_2O (10 mL), dried ($MgSO_4$), and concentrated. Purification of the crude product (120 mg) by chromatography on silica gel (4:1 hexane:ethyl acetate) provided capsidiol 3-phenylthionocarbonate (**13a**) (105 mg, 80%) as a brown oil: TLC R_f = 0.28 (4:1 hexane:ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (m, 2H), 7.28 (m, 1H), 7.10 (m, 2H), 6.10 (dt, 1H, J = 12.3, 4.5 Hz), 5.96 (dd, 1H, J = 6.9, 1.7 Hz), 4.74 (d, 1H, J = 1.5 Hz), 4.70 (d, 1H, J = 0.5 Hz), 4.45 (d, 1H, J = 2.6 Hz), 2.23 (td, 2H, J = 13.4, 4.8 Hz), 2.18 (tt, 1H, J = 10.2, 3.9 Hz), 2.11 (dm, 1H, J = 16.3 Hz), 1.94 (td, 1H, J = 12.9, 3.8 Hz), 1.89 (ddd, 1H, J = 17.0, 5.0, 2.0 Hz), 1.82 (dt, 1H, J = 14.1, 2.8 Hz), 1.74 (s, 3H), 1.46 (s, 3H), 1.36 (app t, 1H, J = 13.8 Hz), 0.96 (d, 3H, J = 6.9 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.0, 153.3, 149.1, 139.6, 129.6, 129.5, 126.4, 122.0, 108.9, 80.5, 74.5, 44.5, 43.5, 40.0, 39.2, 32.4, 31.9, 30.2, 21.0, 10.3; FTIR (neat film) ν 3413.1, 2975.1, 2922.7, 1643.7, 1590.4, 1490.3, 1450.2, 1378.6, 1356.6, 1326.1, 1285.0, 1198.5, 1161.0, 1146.2, 1119.0, 1071.4, 1033.7, 1011.3, 963.4, 918.1, 891.5, 869.9, 824.5, 808.0, 771.8, 740.6, 688.6 cm^{-1}; HRMS (FAB) m/z calcd for $C_{22}H_{28}O_3S$ $(M + H)^+$ 373.1834. Found 373.1834.

3-Deoxycapsidiol (4-Epieremorphila-9,11-dien-1β-ol) (10). The general procedure of Fu was followed.[24] To **13a** (75 mg, 0.20 mmol, 1 equiv) in 4 mL of benzene at room temperature was added a solution of $(Bu_3Sn)_2O$ (10 μL, 0.02 mmol, 0.1 equiv), AIBN (5 mg, 0.03 mmol, 0.15 equiv), and PMHS (120 μL, 2 mmol, 10 equiv) in benzene (4 mL). After 5 min at room temperature, the reaction mixture was heated at reflux for 5 h, cooled to room temperature, and concentrated. THF (5 mL) and aqueous NaOH (2 M, 3 mL) were added. The mixture was stirred for another 12 h at room temperature, and the aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography (6:1 hexane/ethyl acetate) afforded 3-deoxycapsidiol (**10**, 36 mg, 81%) as a pale yellow oil: TLC R_f = 0.20 (6:1 hexane/ethyl acetate); HPLC t_R (14 min, 16 mL/min, 6:1 hexane/ethyl acetate); $[\alpha]^{25}_D$ = −12.7 (c = 0.71, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (dd, 1H, J = 6.8, 2.1 Hz), 4.70 (m, 1H), 4.67 (m, 1H), 4.25 (t, 1H, J = 2.7 Hz), 2.34 (tt, 1H, J = 13.7, 4.7 Hz), 2.14 (tt, 1H, J = 12.1, 3.3 Hz), 2.04 (dddd, 1H, J = 16.2, 6.8, 4.0, 2.8 Hz), 1.86 (ddd, 1H, J = 16.2, 11.4, 2.1 Hz), 1.78 (tt, 1H, J = 14.3, 4.1 Hz), 1.75 (m, 3H), 1.70 (m, 2H), 1.59 (m, 1H), 1.36 (s, 3H), 1.30 (dd, 1H, J = 13.3, 11.1 Hz), 1.29 (s, 1H), 1.19 (ddt, 1H, J = 14.0, 4.3, 2.5 Hz), 0.95 (d, 3H, J = 7.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.8, 142.0, 127.4, 108.5, 74.8, 44.5, 41.2, 40.3, 38.0, 32.6, 30.3, 27.9, 24.8, 21.0, 16.7; FTIR (neat film) ν 3369.4, 2919.1, 2873.1, 1643.2, 1454.7, 1374.7, 1063.5, 1012.1, 950.0, 914.1, 886.4, 819.3 cm^{-1}; HRMS (EI) m/z calcd for $C_{15}H_{24}O$ (M^+) 220.1827. Found 220.1826.

Epiaristolochen-1-one (22). The general procedure of Heathcock was followed.[40] Oxalyl chloride (47 mg, 32 mL, 0.37 mmol) and DMSO (55 mg, 50 mL, 0.71 mmol) were dissolved in CH_2Cl_2 (3 mL), and the solution was cooled to −78 °C. 3-Deoxycapsidiol (**10**) (45 mg, 0.20 mmol) in CH_2Cl_2 (3 mL) was then added slowly. The reaction mixture was stirred at −78 °C for 20 min; triethylamine (145 mg, 200 mL, 1.44 mmol) was added, and the solution was maintained at room temperature for another 20 min. The solution was washed with brine (10 mL); the aqueous layer was extracted with Et_2O (3 × 20 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. Purification of the crude product by flash chromatography (7:1 hexane/ethyl acetate) provided the enone **22** (38 mg, 88%) as a clear oil. The purity of the product was shown to be 99% by GC (program 1) and ^{1}H NMR analyses. Data for **22**: TLC R_f = 0.42 (5:1 hexane/ethyl acetate); GC (99%) (program 1); $[\alpha]^{25}_D$ = −10.6 (c = 1.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.60 (dd, 1H, J = 7.2, 2.2 Hz), 4.74 (quintet, 1H, J = 1.5 Hz), 4.70 (septet, 1H, J = 1.0 Hz), 2.40 (m, 2H), 2.27 (m, 2H), 2.19 (tt, 1H, J = 12.1, 3.8 Hz), 1.93 (ddd, 1H, J = 10.0, 6.4, 2.4 Hz), 1.90 (ddd, 1H, J = 7.4, 3.7, 2.0 Hz), 1.75 (s, 3H), 1.73 (m, 1H), 1.61 (ddt, 1H, J = 13.8, 7.0, 2.3 Hz), 1.35 (dd, 1H, J = 13.9, 13.0 Hz), 1.14 (s, 3H), 1.13 (d, 3H, J = 7.1 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 204.2, 148.7, 143.5, 133.3, 109.3, 42.5, 40.8, 40.6, 39.8, 35.5, 31.4, 30.2, 27.7, 21.0, 16.6; FTIR (neat film) ν 2962.5, 2932.4, 2880.5, 1692.3, 1632.9,

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1456.5, 1376.5, 1258.2, 1177.6, 887.5, 866.9, 810.0 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{15}H_{22}O$ (M^+) 218.1671. Found 218.1667.

Epiaristolochen-1-one Tosylhydrazone (6-Isopropenyl-4,4a-dimethyl-3,4,4a,5,6,7-hexahydro-2*H*-naphthalen-1-tosylhydrazone, 23). The general procedure of Kabalka was followed.[28] A solution of enone **22** (20 mg, 0.09 mmol) in EtOH (4 mL) was heated at 65 °C as tosyl hydrazide (25 mg, 0.13 mmol) was added. The reaction mixture was then heated at reflux for 1 h, cooled to room temperature, and concentrated. Purification by chromatography (6:1 hexane/ethyl acetate) provided tosylhydrazone **23** (21 mg, 55%, containing ~7% hexane observed in the ^{1}H NMR spectrum) as a clear oil: TLC R_f = 0.10 (6:1 hexane/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, 2H, J = 8.3 Hz), 7.31 (d, 2H, J = 7.9 Hz), 6.01 (dd, 1H, J = 7.0, 2.2 Hz), 4.71 (quintet, 1H, J = 1.5 Hz), 4.67 (d, 1H, J = 0.7 Hz), 2.53 (dd, 1H, J = 16.1, 4.3 Hz), 2.43 (s, 3H), 2.20−1.90 (m, 3H), 1.86 (dd, 1H, J = 5.0, 2.3 Hz), 1.80 (dt, 1H, J = 13.9, 3.2 Hz), 1.71 (s, 3H), 1.60 (m, 1H), 1.46 (ddt, 1H, J = 13.6, 6.8, 2.4 Hz), 1.32 (app t, 1H, J = 13.1 Hz), 1.05 (s, 3H), 0.99 (d, 3H, J = 7.1 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.2, 143.9, 138.6, 129.4, 128.0, 108.9, 42.7, 40.4, 40.3, 32.6, 31.6, 30.7, 30.3, 27.3, 22.6, 21.6, 20.9, 16.6, 14.1; FTIR (neat film) ν 3413.1, 3219.9, 2960.9, 2931.8, 1643.1, 1597.9, 1453.8, 1376.9, 1339.3, 1167.2, 1093.8, 968.5, 916.3, 813.7, 680.5 cm^{-1}; HRMS (FAB) *m*/*z* calcd for $C_{22}H_{30}N_2O_2S$ $(M + 1)^+$ 386.2106. Found 386.2104.

(−)-4-Epieremophilene (17). The general procedure of Kabalka was followed.[28] Tosylhydrazone (20 mg, 0.05 mmol) in $CHCl_3$ (1.3 mL) was first degassed by N_2 bubbling for 5 min. The solution was then cooled to 0 °C as catecholborane (40 mL, 1 M in THF, 0.04 mmol) was added. The reaction mixture was stirred for 30 min at 0 °C and warmed to room temperature. $NaOAc{\cdot}3H_2O$ (9 mg, 0.07 mmol) was added, and the mixture was then heated at 65 °C for 50 min, cooled to room temperature, and diluted with pentane (30 mL). After the mixture was washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL), the organic layer was dried ($MgSO_4$) and evaporated. Initial flash chromatography (pentane) provided the product (total yield 7.3 mg, 70%) as a 7:3 mixture of **17** and side-chain-reduced product according to GC−MS. Further purification by chromatography on $AgNO_3$ silica gel (50:1 petane/ether) gave epieremophilene (**17**): yield 5.0 mg (48%); GC (program 2) t_R 30.75 min; TLC R_f = 0.66 ($AgNO_3$ silica gel, 50:1 pentane/ether); $[\alpha]^{25}_D = -22.7$ (c = 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 5.40 (td, 1H, J = 3.7, 1.5 Hz), 4.86 (s, 1H), 4.82 (s, 1H), 2.47 (m, 1H), 2.14 (tt, 1H, J = 8.7, 5.5 Hz), 1.98 (m, 2H), 1.89 (dtt, 1H, J = 17.5, 6.0, 3.5 Hz), 1.72 (m, 1H), 1.68 (s, 3H), 1.66−1.58 (m, 4H), 1.48−1.40 (m, 2H), 1.15 (s, 3H), 0.92 (d, 3H, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.8, 142.3, 120.2, 108.9, 40.2, 40.1, 38.5, 30.2, 29.2, 28.2, 27.1, 23.8, 21.5, 15.6; MS (EI) *m*/*z* 204, 189, 161, 147, 133, 119, 107, 93, 79, 67, 55. GC−MS data for the side-chain-reduced product: GC (program 2) t_R 30.34 min; MS (EI) *m*/*z* 206, 191, 163, 149, 135, 119, 105, 93, 81, 69.

Bioassays. Capsidiol and its derivatives were spotted onto a 1-cm × 1-cm grid marked on silica gel (250 μm) TLC plates at 1, 3, 10, 30, 100, 300, and 1000 ng/spot, and the amounts were verified by GC using (+)-valencene as a standard. Plates were either sprayed with a vanillin indicator reagent (3.5 g of vanillin and 650 μL of H_2SO_4 per 100 mL of methanol, and heated with a hair-dryer for color development) or overlaid with a suspension of *Cladosporium cucumerinum* spores and hyphal fragments. The treated plates were incubated at room temperature in a sealed Tupperware box (11 × 7 × 2.5 in.) containing moistened paper towels for 2−4 days. *C. cucumerinum* was cultivated on clarified V8 agar medium.[41] The resulting hyphal of *C. cucumerinum* was scraped off using a spatula in 10 mL of the medium that contained casamino acid (2 g, acid-hydrolyzed casein with a low concentration of sodium chloride and iron), $ZnCl_2$ (2 mg), $MnSO_4{\cdot}4H_2O$ (2 mg), KCl (110 mg), $MgSO_4{\cdot}7H_2O$ (1.5 g), and glucose (20 g) in 1 L of 2.5 mM KH_2PO_4 (pH 6.0) buffer solution. The suspension was filtered through two layers of miracloth to release the spores and hyphal fragments.

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Supporting Information Available: Details of experimental procedures, characterization data, and reproductions of ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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