Structure and Chemistry of Apicidins, a Class of Novel Cyclic Tetrapeptides without a Terminal α-Keto Epoxide as Inhibitors of Histone Deacetylase with Potent Antiprotozoal Activities

Sheo B. Singh,* Deborah L. Zink, Jerrold M. Liesch, Ralph T. Mosley, Anne W. Dombrowski, Gerald F. Bills, Sandra J. Darkin-Rattray, Dennis M. Schmatz, and Michael A. Goetz

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

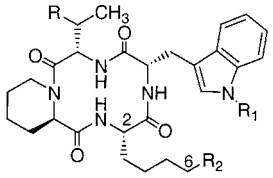
sheo_singh@merck.com

Received September 4, 2001

Apicidins are a class of cyclic tetrapeptides that do not contain the classical electrophilic α-keto epoxide yet are potent (nM) inhibitors of histone deacetylase and antiprotozoal agents. These compounds showed broad-spectrum activities against the apicomplexan family of protozoa including *Plasmodium* sp (malarial parasite), *Toxoplasma gondii*, *Cryptosporidium* sp., and *Eimeria* sp. These cyclic peptides contain a *β*-turn amino acid (*R)*-Pip or (*R)*-Pro, (*S*)-*N*-methoxy Trp, (*S*)-Ile, or (*S*)-Val, and either (*S*)-2-amino-8-oxodecanoic acid or a modified (*S*)-2-amino-8-oxodecanoic acid. The isolation and structure elucidation of new apicidins from two *Fusarium* species, temperature-dependent NMR studies of apicidin, NMR and molecular modeling based conformation of the 12-membered macrocyclic ring, and selected chemical modifications of apicidin have been detailed in this paper. The cyclic nature of the peptide, the C-8 keto group, and the tryptophan are all critical for the biological activity.

Malaria is a life-threatening disease caused by infections with *Plasmodium* sp., a member of the apicomplexan protozoan family. It remains one of the leading causes of loss of life worldwide, accounting for over 1.5 million deaths annually.[1] Members of this family of protozoa cause numerous diverse human and animal diseases notably cryptosporidosis, toxoplasmosis, sarcocystis, and coccidiosis caused by *Cryptosporidium parvum*, *Toxoplasma gondii*, *Sarcocystis neurona*, *Eimeria* sp., respectively. Outbreaks of cryptosporidiosis are associated with opportunistic infections spread through contaminated water supplies and causes significant health crises in certain parts of the world.[2] There is no treatment regimen available to manage this disease. Cryptosporidiosis along with toxoplasmosis pose serious health problems in immune-compromised individuals. Needless to say that effective therapies are sorely needed to manage these diseases. In animal health, a number of anticoccidials are available for prophylactic control of coccidiosis caused by *Eimeria* sp.; however, continual administrations of these agents are causing serious resistance. Therefore, superior therapeutic agents with newer mechanism of actions are needed to control outbreaks of coccidiosis.

Screening for anticoccidial agents using the cultured Apicomplexan protozoa led to the discovery[3] of apicidin (**1a**), the first member of the novel cyclic tetrapeptide series. Apicidin consists of (*R*)-Pip, (*S*)-Ile, (*S*)-*N*-methoxy-Trp, and the new amino acid (*S*)-2-amino-8-oxodecanoic acid (Aoda) residues.



Compound	R	R_1	R_2	Name
1a	CH_2CH_3	OCH_3	$CH_2COCH_2CH_3$	Apicidin
1b	CH_2CH_3	H	$CH_2COCH_2CH_3$	Apicidin A
1c	CH_3	OCH_3	$CH_2COCH_2CH_3$	Apicidin C
1d	CH_2CH_3	OCH_3	$CH_2COCH(OH)CH_3$	Apicidin D_1
1e	CH_2CH_3	OCH_3	$CH_2CH(S\text{-}OH)CH_2CH_3$	Apicidin D_2
1f	CH_2CH_3	OCH_3	$CH_2CH_2CH(OH)CH_3$	Apicidin D_3

Apicidin showed broad-spectrum activity[4] and potently inhibited the growth of many members of the apicomplexan family. Subsequently, we elucidated the mechanism by which this peptide exerts its biological activity. It *reversibly* blocked the activity of histone deacetylase (HDAC),[4,5] a nuclear isozyme that in conjunction with histone acetylase (HAT) regulates gene transcription by controlling the dynamic process of acetylation and deacetylation of lysine residue (Figure 1).[5] Blockade of the process of deacetylation causes hyperacetylation[5] of histones and unregulated gene activation that results in untimely cell death.

* To whom all correspondence should be addressed. Fax: (+1) 732-594-6880.

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10.1021/jo016088w CCC: $22.00
Published on Web 01/12/2002

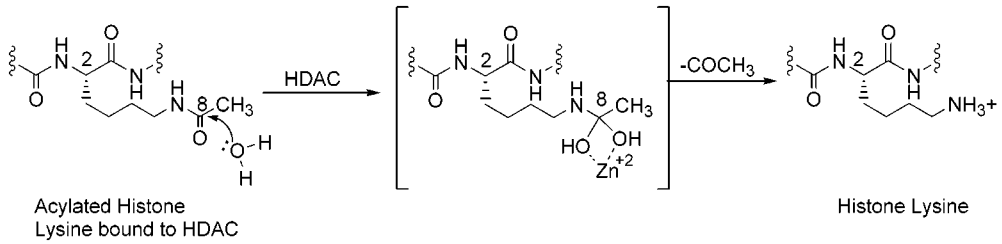
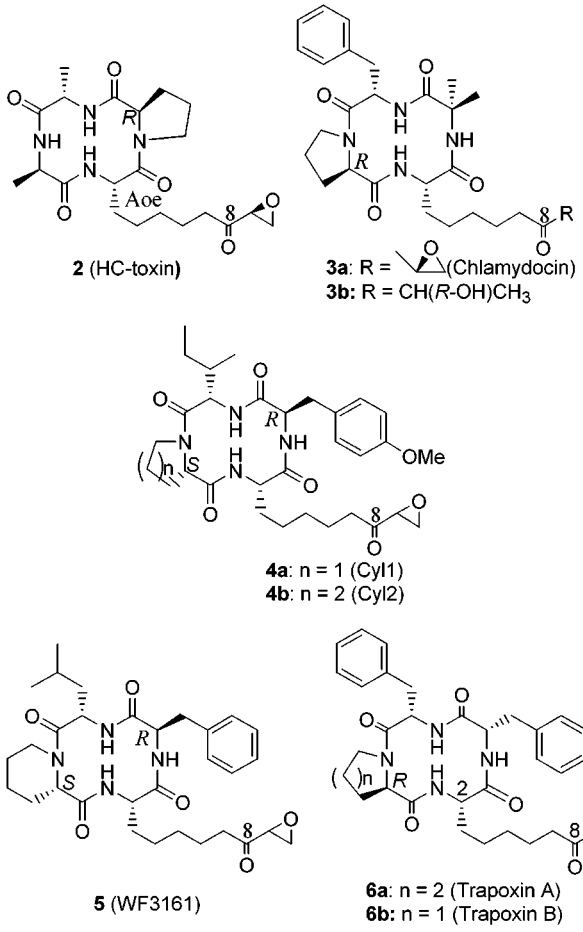


Figure 1. Amide hydrolysis catalyzed by histone deacetylase.

A small group of natural fungal cyclic tetrapeptides has been reported as antiproliferative or phytotoxic agents. These include HC-Toxin I (**2**),[6] chlamydocin (**3a**)[7] and analogue **3b**,[8] Cyl-1 (**4a**) and Cyl-2 (**4b**),[9] WF-3161 (**5**),[10] and trapoxins A (**6a**) and B (**6b**).[11] Like apicidin, these peptides contain a β-turn amino acid (Pro or Pip), and at least one of the amino acids has a D configuration, but unlike apicidin, all but **3b** contain a (2*S*,9*S*)-2-amino-8-oxo-9,10-epoxydecanoic acid (Aoe) residue with a highly electrophilic terminal α-keto epoxide. The acid group of the β-turn amino acid is coupled to the amino group of Aoe in all the natural cyclic peptides (Aoda in apicidin) except HC-Toxin where it is reversed.



The potent antiproliferative and phytotoxic activities of these compounds have been exclusively associated with the α-keto epoxide moeity. Compound **3b** is the only exception, but its phytotoxic activity is rather weak (μM).[8] Trapoxin A (**6a**)[12] and HC-Toxin[4,13] have been reported as irreversible inhibitors of HDAC. Like antiproliferative activity, the elimination of the epoxy group of HC-Toxin led to the complete loss of HDAC activity.[13] The potent HDAC inhibitory activity of apicidin is therefore surprising. It appears that the C-8 keto group of Aoda topologically registers to the C-8 keto group of the acetylated lysine residue of histones and suggests that apicidin functionally mimics the substrate for HDAC thus causing hyperacetylation.[4] Tryptophan and other aromatic residues connected to the Aoda residue appear to have an additive effect on the potency.[13] Apicidin showed in vivo efficacy against *P. berghei* malaria in mice.[4] Extensive chemical modifications of Aoda and Trp residues of apicidin led to critical SAR.[14] However, in the absence of a total synthesis, the impact of the amino acid substitutions on the SAR could not be directly evaluated. To study the impact of the amino acid substitutions on the SAR, we decided to perform a targeted analytical and biological screening of fermentation extracts in order to find congeners of apicidin. This led to the isolation of a number of congeners including apicidin B **7a** (Pip → Pro), apicidin C **1c** (Ile → Val), and Aoda modified apicidins D_1 (**1d**), D_2 (**1e**), and D_3 (**1f**). Detailed isolation of all

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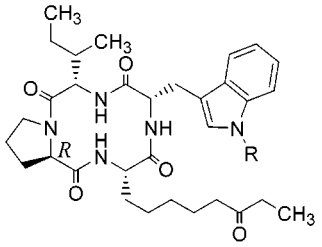
apicidins from two different fungal species, structure elucidation and stereochemistry of new congeners, conformation of the 12-membered macrocyclic ring of the cyclic peptide based on NMR spectroscopy including temperature-dependent chemical shifts of the amide protons of apicidin, molecular modeling, and selected chemical modification including ring opening by transesterification are described.

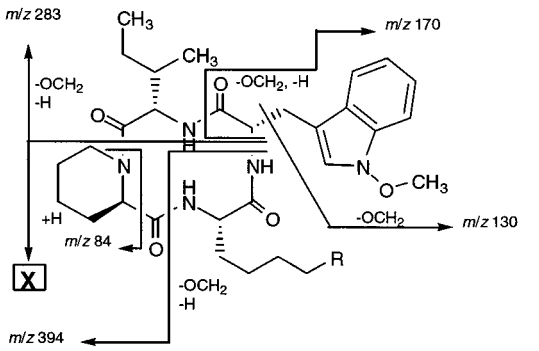


7a: R = OCH_3 (Apicidin B)
7b: R = H

Results and Discussions

Isolation of Apicidins. The fungus *Fusarium pallidoroseum* (ATCC 74289) was grown on a vermiculite-based solid medium. It was extracted with methyl ethyl ketone, and the extract was chromatographed on Sephadex LH 20 in MeOH. The fractions containing mixture of apicidins were pooled and partially purified by two successive chromatographies on silica gel which gave four fractions (I−IV). Trituration of fraction I with MeOH followed by recrystallization from MeOH afforded apicidin **1a** (35.7 mg/L). Reverse phase HPLC of fraction II gave apicidin C **1c** (0.9 mg/L), III gave apicidins B **7a** (0.5 mg/L), D_2 **1e** (0.18 mg/L), and A **1b** (0.18 mg/L), and IV yielded apicidin D_1 **1d** (0.9 mg/L). Apicidin D_3 **1f** (0.12 mg/L) eluted between fractions III and IV and was isolated from a larger (10 L) fermentation batch following similar isolation protocols. A second strain (*Fusarium* sp., ATCC 74322) was found to produce more apicidin **1a** (>100 mg/L in solid vermiculite medium), but after optimization of the fermentation conditions in liquid medium[15] it produced up to 1.2−2 g/L. This strain always produced all the apicidins with the exception of proline-containing apicidin B (**7a**). Apicidin C (**1c**) predominated among the apicidin minors, typically amounting to 5% of the apicidin (**1a**) content, occasionally reaching 10%; apicidins A (**1b**), D_1 (**1d**), D_2 (**1e**), and D_3 (**1f**) were present in amounts ranging from 0.3 to 1% of apicidin.

Apicidin (1a), Apicidin A (1b), Apicidin B (7a), and Apicidin C (1c). The structure and stereochemistry of apicidin **1a** and apicidin A **1b** was elucidated by application of 2D NMR spectroscopy, acid hydrolysis followed by derivatization with α-methylbenzyl isothiocyanate (AMBI) and L-amino acid oxidase, HPLC analysis of amino acid derivatives, NOESY spectroscopy, and molecular modeling.[3] The structure of apicidin B (**7a**), desmethoxy **7b**, and apicidin C **1c** was also recently reported.[16] The EIMS spectrum (Figure 2) of apicidin exhibited a number of distinctive fragment ions which were critical for the identification of the new congeners. For example, it showed two fragment ions at *m/z* 283 and



Compound	R	Parent ion (*m/z*)	X (*m/z*)
1a	$CH_2COCH_2CH_3$	623	310 and 312
1d	$CH_2COCH(OH)CH_3$	639	326
1e	$CH_2CH(S\text{-}OH)CH_2CH_3$	625	312 and 314
1f	$CH_2CH_2CH(OH)CH_3$	625	312 and 314

Figure 2. EIMS fragmentation pattern of apicidins.

m/z 310/312 resulting from splitting the tetrapeptide molecule into two halves, an upper and a lower half, respectively. The third major critical fragment ion exhibited by the mass spectrum appeared at *m/z* 394 consisting of three amino acids (Trp-Ile-Pip). As expected, substitution of Pip with Pro in apicidin B (**7a**) caused a Δ14 Da shift (*m/z* 310/312 → *m/z* 296/298 and *m/z* 394 → *m/z* 380) of the two of the fragment ions involving these amino acids. Similarly, the substitution of Ile → Val in apicidin C (**1c**) produced Δ 14 Da shift (*m/z* 283 → *m/z* 269 and *m/z* 394 → *m/z* 380) in the two fragment ions reflecting the corresponding changes. The EIMS of apicidin (**1a**) showed a fragment ion for Pip at *m/z* 84 which was shifted to *m/z* 70 in the EIMS of apicidin B (**7a**) reflecting the Pro substitution.

Stereochemistry and Conformation of Macrocyclic Ring. The stereochemistry of apicidin (**1a**) was determined by comparison of the AMBI derivatives of amino acids derived from the hydrolysis of apicidin with the derivatives of corresponding standard amino acids, and by NOESY analysis.[3] The 12-membered macrocyclic ring is highly rigid due to a network of three hydrogen bonds via seven-membered rings employing Ile-NH−Aoda-CO, Aoda-NH−Ile-CO, and Trp-NH−Pip-CO, and this was clearly evident from the concentration (1−10 mM) independent chemical shifts of the amide protons of apicidin and further substantiated by the NOESY correlation of Ile-NH with Aoda-NH in the ^{1}H NMR spectrum (Table 1).[3] To further verify this network of intramolecular H-bonding, we studied the effect of temperature gradient on the ^{1}H NMR chemical shifts of apicidin protons. Spectra were recorded in pyridine-d_5 at 10 °C intervals beginning with −10 °C and ending at 70 °C. The effect of the temperature on the chemical shifts of the amide protons together with the residual water is depicted in Figure 3. The slope of the curve ($\Delta\delta$) and temperature coefficient ($\Delta\delta/\Delta T$ in ppb/°C)[17] of these and a number of selected protons are presented in Table 2. As expected, the chemical shift of residual water proton was most affected by the temperature gradient as indi-

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Table 1. ^{1}H and ^{13}C NMR Assignments of Apicidins D_1, D_2, and D_3 in C_5D_5N

	apicidin (**1a**)			apicidin D_1 (**1d**)		apicidin D_2 (**1e**)		apicidin D_3 (**1f**)	
no.	δC	mult	δH	δC	δH	δC	δH	δC	δH
					Pip				
1	172.90	CO		172.07		172.08		172.10	
2	51.35	CH	5.53, brd, 5.6	50.97	5.50, brd, 5.6	50.99	5.54, brd, 5.5	50.98	5.51, brd, 6
3	25.14	CH_2	2.10, m	23.55	2.05, brd, 14	24.73	2.04, brd, 13.6	24.76	2.05, brd, 14.0
			1.48, m		1.45, m		1.48, m		1.45, m
4	20.44	CH_2	2.32, m	20.28	2.30, m	20.06	2.32, m	20.07	2.31, m
			1.44, m		1.45, m		1.44, m		1.40, m
5	26.25	CH_2	1.25, m	25.92	1.45, m	26.04	1.50, m	25.87	1.48, m
			1.50, m		1.25, m		1.30, m		1.30, m
6	44.64	CH_2	4.37,brd, 12.0	44.26	4.35, d, 12.8	44.29	4.38, brd, 13.5	44.27	4.34, d, 13.5
			3.27, dt, 13.2, 2.4		3.24, dt, 11.2, 2.0		3.27, dt, 13.2, 2.0		3.25, dt, 11, 2.0
					Ile				
1	174.90	CO		174.50		174.53		174.54	
2	54.87	CH	5.18, t, 10	54.47	5.16, t, 10.4	54.44	5.19, t, 10.0	54.49	5.16, t, 10.0
3	35.85	CH	2.42, m	35.47	2.40, m	35.48	2.42, m	35.48	2.40, m
4	25.59	CH_2	1.90, m	25.20	1.84, m	25.19	1.85, m	25.87	1.84, m
			1.40, m		1.35, m		1.40, m		1.35, m
5	11.40	CH_3	0.94, t, 7.2	11.00	0.91, t, 7.6	11.01	0.94, t, 7.6	11.02	0.91, t, 8.0
6	16.26	CH_3	1.00,d, 7.2	15.87	0.97, d, 6.8	15.86	1.00, d, 6.8	15.88	0.97, d, 6.5
	- - - -	NH	8.27, d, 10.4	NH	8.26, d, 10.0	NH	8.28, d, 10.0	NH	8.27, d, 10.5
					Trp-N-OCH_3				
1	175.25	CO		174.86		174.90		174.90	
2	62.08	CH	4.61, td, 10.6, 6.4	61.57	4.58, dt, 10.4, 6.4	61.35	4.61, td, 10.4, 6.4	61.64	4.61, td, 10.5, 6.5
3	26.47	CH_2	4.23, dd, 14.4, 10.4	26.11	4.20,dd,14.4, 10.4	25.86	4.23, dd,14.4,10.4	26.29	4.20, dd,14.5,10.5
			3.84, dd, 14.4, 6.4		3.82,dd,14.4,6.4		3.86, dd, 14.0, 6.0		3.82, dd, 14.5, 6.5
4	108.72	CO		108.32		108.30		108.34	
5	124.60	CO		124.22		124.20		124.23	
6	119.86	CH	7.79, d, 8.0	119.48	7.76, d, 8	119.48	7.79, d, 7.6	119.50	7.76, d, 8
7	120.63	CH	7.18 dt, 8.0, 0.8	120.25	7.16, t, 7.2	120.26	7.19, t, 8.0	120.26	7.16, t, 7.5
8	123.28	CH	7.34, dt, 8.0, 0.6	122.90	7.30, t, 8	122.88	7.34, t, 7.2	122.90	7.31, t, 8.0
9	109.32	CH	7.55, d, 8.0	108.94	7.51, d, 8	108.70	7.55, d, 8.4	108.94	7.52, d, 8.5
10	133.38	CO		133.38		133.00		133.00	
11	123.31	CH	7.58, s	122.93	7.56, s	122.94	7.58, s	122.94	7.55, s
12	66.10	CH_3	3.94, s	65.71	3.92, s	65.70	3.94, s	65.69	3.91, s
		NH	10.00, d, 6.8	NH	9.95, d, 6.4	NH	10.05, d, 6.4	NH	10.00, d, 7.0
					Aoda				
1	177.00	CO		176.53		176.63		176.60	
2	55.28	CH	4.76, brq, 8.0	54.97	4.72, brq, 7.2	55.14	4.79, brq, 8.4	55.00	4.76, brq, 8.5
3	30.71	CH_2	1.80, m	30.33	1.84, m	30.50	1.93, m	30.47	1.90, m
			1.60, m		1.60, m		1.65, m		1.60, m
4	26.25	CH_2	1.25, m	25.86	1.25, m	29.69	1.30, m	29.50	1.20, m
5	29.32	CH_2	1.10, m	29.06	1.10, m	26.19	1.10, m	26.06	1.10, m
6	24.22	CH_2	1.45, m	23.55	1.50, m	26.19	1.50, m	26.12	1.50, m
7	42.44	CH_2	2.18, t, 7.2	37.50	2.56, m	37.71	1.45, m	24.35	1.30, m
8	210.77	CO		213.87		72.14	3.66, m	40.14	1.60, m; 1.40, m
9	36.04	CH_2	2.28, q, 7.2	73.41	4.43, m	31.00	1.60, quintet, 7.6	66.97	3.94, m
				OH	6.70, d, 4.4	OH	5.51, d, 5.5	OH	5.65, d, 5
10	8.40	CH_3	1.00, t, 7.6	20.05	1.44, d, 6.8	10.59	1.09, t, 7.6	20.06	1.31, d, 6.0
		NH	7.36, d, 10	NH	7.32, d, 10.0	NH	7.37, d, 9.6	NH	7.34, d, 10.0

cated by the largest temperature coefficient of −15.38 ppb/°C. The amide protons were affected to a moderate extent with coefficients of −4.0, −5.13, and −9.25 ppb/°C for Ile-NH, Aoda-NH, and Trp-NH, respectively. The nonexchangeable protons were least affected with coefficients ranging from −0.63 to 1.38 ppb/°C. In contrast to the amide protons, all nonexchangeable protons showed positive coefficients except for three of four α-protons. Only Aoda α-proton showed a positive coefficient *albeit* a small one (+0.13 ppb/°C). The difference in the behavior of the α-protons could be due to the vicinal polar groups. Among the amide protons, Ile-NH was least affected (temp coefficient = −4 ppb/°C), and this is indicative of stronger H-bonding and a shorter distance.[17] This observation was strongly supported by molecular modeling studies. The energy minimization and molecular dynamics calculations of apicidin after introducing observed NOE constraint generated molecular models consistently exhibiting shorter interatomic distance (2.0−2.6 Å) for Ile-NH and Aoda-CO than the other two H-bonded amide pairs, Aoda-NH−Ile-CO (2.1−3.2 Å) and Trp-NH−Pip-CO (2.2−4 Å). These observations corroborated the rigidity of the 12-membered ring of cyclic tetrapeptide. The substitutions of acyclic amino acids do not have any effect on the conformation of the macrocyclic ring structure as indicated by the virtually superimposable ^{1}H NMR shifts of the corresponding amide and the α-protons and molecular models (Figure 4) of apicidin and apicidin C.[16] This is also true for the remaining apicidins D_1−D_3 (vide infra). However, substitution of the *β*-turn amino acid (Pip → Pro) caused modest chemical shift changes of the protons in the vicinity (cf. *δ* 0.6 ppm downfield shift of Aoda NH, *δ* 0.4 and *δ* 0.25 ppm upfield shifts of α-protons of Pro and Ile)[16] without causing much effect on the chemical shifts of the distal including amide protons. This modest conformational difference was also evident from the overlaying of the molecular models of apicidin, apicidin B, and apicidin C (Figure 4). Molecular modeling of apicidin B generated two equivalent conformers (Figure 4) with identical conformation of the

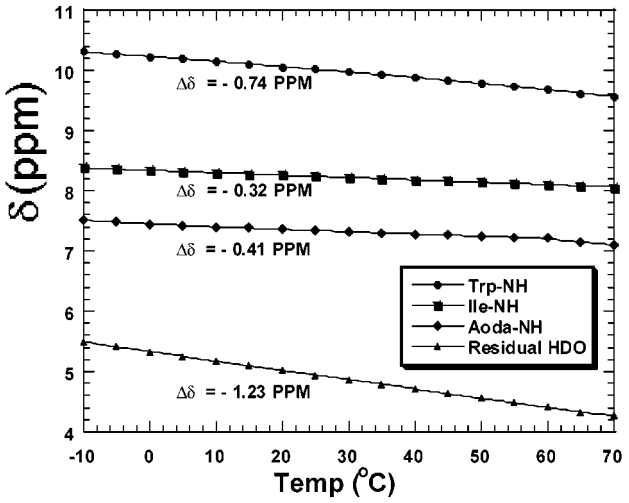


Figure 3. ^{1}H NMR chemical shifts of amide protons of apicidin recorded at 10 °C intervals from −10 °C to 70 °C (ΔT = 80 °C) in pyridine-d_5.

Table 2. Effect of 80 °C Temperature Gradient on Differential ^{1}H NMR Chemical Shifts and Temperature Coefficient

protons	Δδ (ppm)	temp coeff[a]
Trp-NH	−0.74	−9.25
Trp-H2	−0.06	−0.75
Trp-NOMe	0.11	1.38
Trp-H11	0.05	0.63
Ile-NH	−0.32	−4.00
Ile-H2	−0.06	−0.75
Ile-H6	0.08	1.00
Pip-H2	−0.05	−0.63
Aoda-NH	−0.41	−5.13
Aoda-H2	0.01	0.13
Aoda-H10	0.10	1.25
HDO	−1.23	−15.38

[a] Temperature coefficient ($\Delta\delta/\Delta T \times 1000$) ppb/°C.

macrocyclic ring with only slight variation at the level of the five-membererd Pro ring. These models indicate that the Trp-NH and Pip-CO are on the β-face while the Ile-NH, Aoda-NH, Aoda-CO, and Ile-CO are on the α-face with alkyl groups of the amino acids occupying the respective pseudoequatorial positions on the α-face. These experiments provide additional support for the strong H-bonding and high rigidity of the bis-γ-turn macrocyclic structure. The solution conformation of the cyclic tetrapeptide is similar to the X-ray derived conformation of cyclic tetrapeptides, dihydrochlamydocin,[18] and trapoxin A.[11,19]

Chemistry of Apicidin. Sodium borohydride reduction of apicidin gave a 1:1 mixture of inseparable diastereomeric alcohols **8** in >85% yield. Acetylation of **8** produced diastereomeric acetates **9**. Reaction of **8** with methanesulfonyl chloride produced mesylate **10** which upon β-elimination with DBU produced an inseparable mixture of olefins **11** that was hydrogenated to give desmethoxydeoxyapicidin **12** (Scheme 1). The reaction of apicidin with *O*-benzylhydroxylamine afforded a mixture of two isomeric oximes **13**. The Pip−Ile amide bond of the 12-membered ring of the cyclic tetrapeptide was selectively cleaved under transesterification conditions. Thus, the reaction of apicidin **1a** with EtOH and MeOH

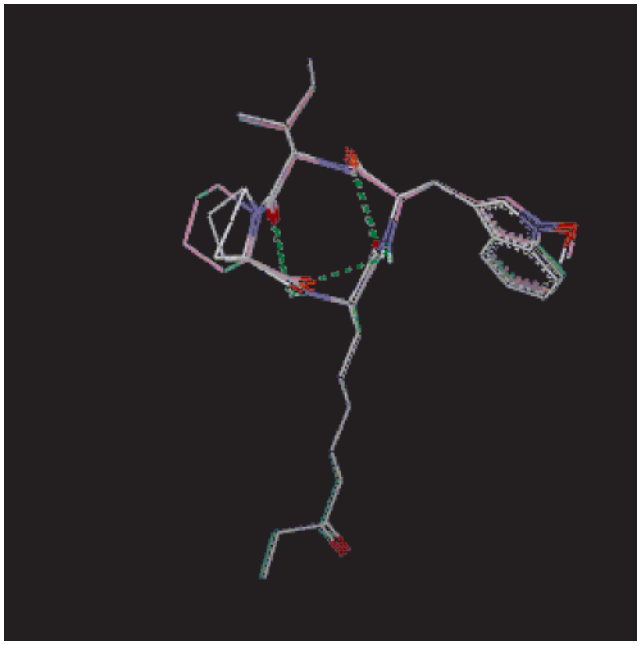
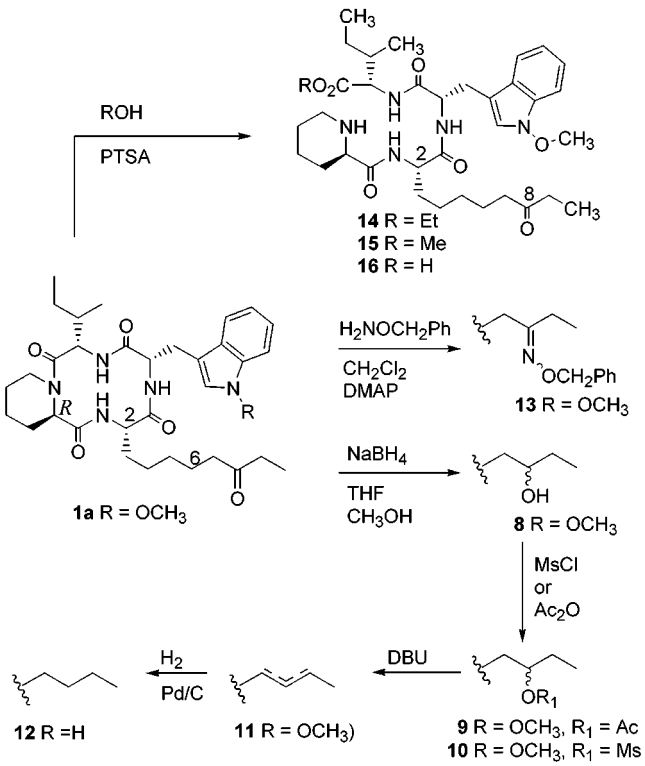


Figure 4. Superimposed 3D energy minimized molecular model of apicidins (**1a**, **1c**, and **7a**) using NOE constraints of apicidin.

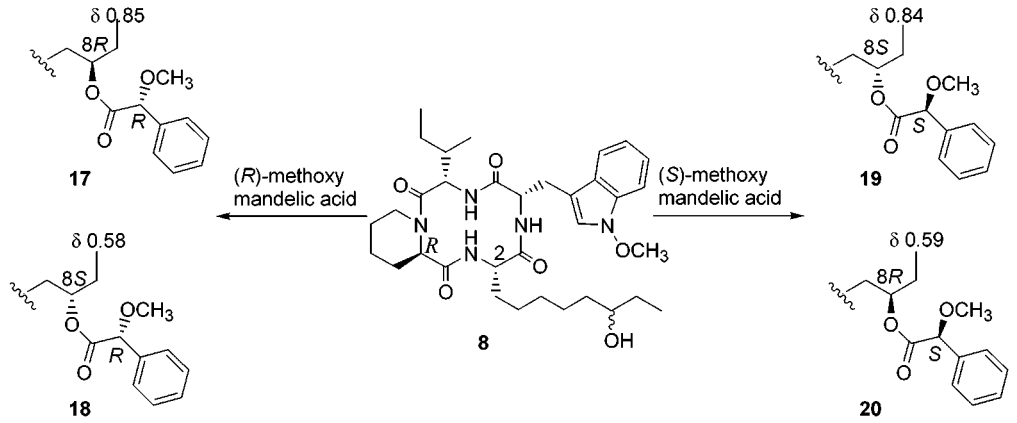
Scheme 1



in the presence of PTSA or HCl furnished exclusively the esters **14** and **15**, respectively. Hydrolysis of ethyl ester **14** with LiOH produced the desired acid **16**. Attempted cyclization of acid **16** using the conditions (BOP reagent in DMF) employed for the cyclization of trapoxin B[12b] was not successful although a similar cyclization of Pro-NH with Phe-CO_2H has been successfully applied in the synthesis of chlamydocin analogues.[20] The steric bulk of Ile appears to prevent the cyclization of **16**.

(18) Flippen, J.; Karle, I. L. *Biopolymers* **1976**, *15*, 1081.
(19) Nakai, H.; Nagashima, K.; Itazaki, H.; *Acta Crystallogr. Sect. C. Cryst. Struct. Commun.* **1991**, *C47*, 1496.
(20) Shute, R. E.; Dunlap, B.; Rich, D. H. *J. Med. Chem.* **1987**, *30*, 71.

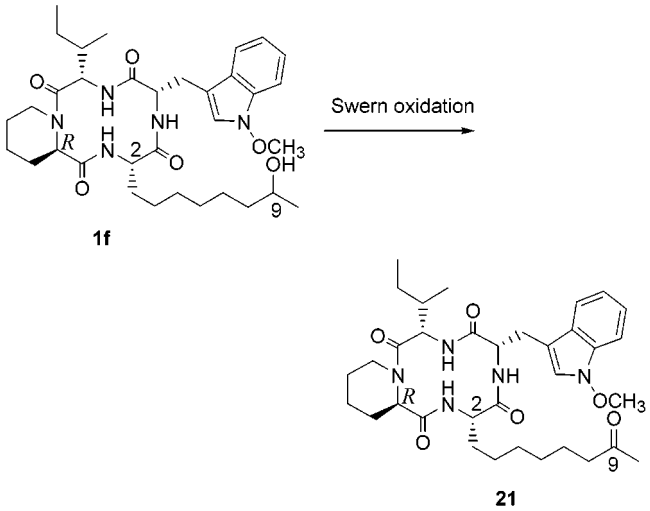
Scheme 2



Apicidin D_1 (1d). EIMS of apicidin D_1 gave a molecular ion at *m*/*z* 639. High-resolution mass measurement led to a molecular formula $C_{34}H_{49}N_5O_7$, which was corroborated by the ^{13}C NMR spectrum in C_5D_5N (Table 1) and suggested 13 degrees of unsaturation, as in apicidin **1a**. The infrared and UV spectra of **1d** were similar to those of apicidin and showed absorption bands for hydroxyl, carbonyl, amide carbonyls, and indole moieties. The molecular formula of **1d** indicated that it was a hydroxylated apicidin. The mass spectrum of apicidin D_1 showed two of the common fragment ions at *m*/*z* 283 and 394 as observed for apicidin. The third fragment ion (X) was shifted by 16 Da and appeared at *m*/*z* 326, indicating the site of hydroxylation to be in the Aoda residue. The ^1H and ^{13}C NMR spectra of **1d** were identical to the corresponding spectra of apicidin including NH shifts, except for the appearance of a new methyl doublet (δ_H 1.44, δ_C 20.05) and a methine multiplet (δ_H 4.43, δ_C 73.41) in apicidin D_1 in place of the terminal ethyl group of Aoda. Thus, apicidin D_1 is 9-hydroxyapicidin.

Apicidin D_2 (1e). High-resolution EIMS of **1e** revealed a molecular formula $C_{34}H_{51}N_5O_6$ that was supported by the ^{13}C NMR spectrum in C_5D_5N (Table 1) and suggested 12 degrees of unsaturation. The formula of **1e** contained two additional hydrogens compared to apicidin, indicating it to be a dihydro derivative. The infrared and UV spectra of **1e** were similar to those of apicidin and showed absorption bands for hydroxyl, amide carbonyls, and indole moieties. Like apicidin D_1, the mass spectrum of apicidin D_2 showed two of the common fragment ions at *m*/*z* 283 and 394 as observed in the spectra of apicidin and apicidin D_1. However, the third fragment ion (X) was shifted by 2 Da and appeared at *m*/*z* 312/314, indicating reduction of the C-8 ketone. The ^1H and ^{13}C NMR spectrum of **1e** were essentially identical to the corresponding spectra of **1a** including NH shifts and other Pip containing apicidins except for the absence of the signals for the ethyl ketone and appearance of a methine multiplet (δ_H 3.66, δ_C 72.14), a methylene quintet (δ_H 1.60, δ_C 31.00), and retention of the methyl triplet (δ_H 1.09, δ_C 10.59). Based on these spectral data, the structure for apicidin D_2 was elucidated as dihydroapicidin (**1e**). The stereochemistry of the C-8 chiral center was established by modified Mosher method. Due to limited supply of apicidin D_2, we chose to use epimeric dihydroapicidin **8** for the preparation of Mosher esters. Therefore, diastereomeric alcohol **8** was reacted with both *R* and *S* methoxymandelic acid to produce 8*R*,*R* (**17**), 8*S*,*R* (**18**) and 8*S*,*S* (**19**), 8*R*,*S* (**20**) esters, respectively (Scheme 2). The NMR spectra of these compounds were carefully assigned by 2D NMR spectroscopy (COSY, TOCSY), and stereochemistry was deduced by applying differences ($\Delta\delta = \delta S - \delta R$) of the corresponding chemical shifts. Reaction of apicidin D_2 (**1e**) with *R*-methoxymandelic acid afforded an ester, which was identical to compound **18**, thus establishing *S*-absolute stereochemistry at C-8 of Aoda and also confirming the structure of apicidin D_2 as dihydroapicidin (**1e**).

Scheme 3



Apicidin D_3 (1f). High-resolution EIMS of **1f** revealed a molecular formula $C_{34}H_{51}N_5O_6$ and indicated that it was isomeric to apicidin D_2. The molecular formula was corroborated by the ^{13}C NMR spectrum in C_5D_5N (Table 1). The infrared and UV spectra of **1f** were also similar to those of apicidin D_2. The mass spectrum of apicidin D_3 was similar to the mass spectrum of apicidin D_2 and showed two of the common fragment ions at *m*/*z* 283 and 394. Like that of apicidin D_2, the mass spectrum of apicidin D_3 showed X fragment ions at *m*/*z* 312 and 314, thus suggesting the isomeric nature of the compounds. The ^1H and ^{13}C NMR spectrum of **1f** was identical to both apicidin (**1a**) and apicidin D_2 (**1e**) except that it showed a deshielded methyl doublet (δ_H 1.31, δ_C 20.06) like in **1d**. In addition, it showed signals for a shielded methine (δ_H 3.94, δ_C 66.97) like in **1e** but not those for the C-8 keto group, thus establishing the structure of **1f** as 9-hydroxy-8-deoxyapicidin (**1f**). Swern oxidation of apicidin D_3 produced C-9 keto derivative **21** in excellent yield (Scheme 3).

Biological Activity. The biological activities of apicidins and the analogues were evaluated in four assays.[4]

Table 3. Biological Activities of Apicidins and Analogs

	HDAC (IC_{50}, nM)		^{3}H-Apicidin A binding (IC_{50}, nM)		whole cell (nM)	
compound	*E. tenella*	Hela	*E. tenella*	chick liver	*E. tenella* (MIC)	*P. falciparum* (IC_{50})
1a	1	1	4	4	93	150
1b	1	2	4	4	52	59
1c	-	-	6	6	101	69
1d	4	0.3	20	2	13	34
1e	400	400	800	900	800	1600
1f	-	-	2000	>1000	400	184
7a	-	-	10	50	411	189
8	-	-	1000	600	>1600	-
9	-	-	>1000	>1000	1500	1000
12	-	-	>1000	>1000	1728	>1728
13	700	>1370	4000	4000	1374	426
14	-	-	-	-	>1495	>1495
15	-	-	-	-	>1527	>1527
17	-	-	>5000	>5000	1295	920
18	-	-	>5000	>5000	>1295	466
19	-	-	>5000	>5000	>1295	350
20	-	-	>10000	>10000	>1295	324
21	-	-	>1000	>1000	>1605	>1605

(I) HDAC enzyme inhibition assay using partially purified HDAC from extracts of human Hela cells and from *E. tenella* cells reported as IC_{50} values. (II) Measurements of ^{3}H-apicidin A binding to a crude preparation of HDAC from chick liver (host) and *E. tenella* (parasite) also reported IC_{50} values. (III) An in vitro functional assay using whole red blood cells infected with *P. falciparum* also reported as IC_{50} values. (IV) An in vitro functional assay using MDBK (Mauden-Downing bovine kidney) host cells infected with *E. tenella* and reported as MIC (minimum inhibitory concentration) values. The data originating from these assays are presented in Table 3. Apicidin (**1a**), apicidin A (**1b**), and apicidin D_1 (**1d**) are among the most active compounds of the series. Unfortunately, these compounds did not show any parasite selectivity as evidenced by similar potencies against Hela and *E. tenella* HDAC as well as host and parasite HDAC binding activities. Early on in the program it was found that apicidin D_2 and compound **8** (C-8 hydroxy compound) were over 10-fold less active in functional assays compared to apicidin that provided the key evidence, suggesting the critical role played by the keto group. This was substantiated by 200- and 400-fold loss of activity in binding and enzyme assays, respectively. Acetylation of the hydroxy group (compound **9**) did not improve the activity. This observation led to the formulation of the idea and eventual elucidation of the HDAC inhibitory mechanism of this class of compounds. The observation was further substantiated by the essentially complete loss of the activities in compounds **12** and **13**; further evidence came from additional chemical modifications and SAR of the side chain.[14d] The α-hydroxy keto containing compound apicidin D_1 (**1d**) is somewhat more potent across all the assays and turned out to be least selective of the series presumably due to its ability to better chelate the bivalent ions which are required for HDAC function. The activity of **1e** appears to be independent of the stereochemistry of the C-9 hydroxy group as the racemate was equally active.[14d] The substitution of Ile to Val (**1c**) had very little effect on binding and functional activities. However substitution of Pip to Pro (**7a**) caused 2−3-fold loss of the binding activity against *E. tenella* with a 5-fold gain in selectivity against chick liver. The difference in activity of apicidin B compared to apicidin could be explained due to a slight backbone conformational difference caused by the Pro substitution (Figure 4). Like apicidin D_2 (**1e**), apicidin D_3 (**1f**), which has a hydroxy group at the C-9 group, was equally less active than apicidin. Oxidation to C-9 keto (cf. compound **21**) did not improve potency, confirming that the exact location of the C-8 keto group is critical for proper mimicking of acetylated histone lysine.[14d] Other modifications to apicidin caused complete loss of the activities (cf., compounds **12**−**20**). The chemical modifications of the tryptophan residue led to the compounds that showed significant parasite selectivity.[14e]

In summary, we have discovered a series of novel cyclic tetrapeptides that show a variety of potent antiprotozoal activities by reversibly inhibiting HDAC. The C-8 keto group of apicidin is critical for the activity of this class of compounds, and this has been confirmed by the lack of activities of the keto-reduced congeners. We have also demonstrated that the exact location of the keto group is equally important. These studies have led us to define a minimum pharmacophore model for apicidin in which a cyclic tetrapeptide with C-8 Aoda keto group and tryptophan or another aromatic amino acid next to the Aoda residue is required for activity.[14] The congener with a α-hydroxy keto group (apicidin D_1) showed better activity presumably due to better binding to bivalent metal ions, but this feature caused it to be less parasite selective. These unique cyclic tetrapeptides do not require the electrophilic α-keto epoxide moiety of HC-Toxin, trapoxin, or chlamydocin to be active against HDAC and the apicomplexan protozoal parasites. Further studies on the apicidins may lead to better antimalarial and antiprotozoal agents.

Experimental Section

General Procedure. Reagents were obtained from Sigma-Aldrich. All solvent extracts were dried on anhydrous Na_2SO_4. NMR spectra were recorded on Varian Inova 400 or 500 MHz instruments operating at 400 and 500 MHz for ^{1}H and 100 and 125 MHz for ^{13}C nuclei. An HP1100 was used for analytical HPLC. LC-MS was performed on a Thermo Quest LCQ instrument using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Mass spectra were recorded on JEOL SX-102A (electron impact, EI, 90 eV). High-resolution mass spectral analyses were performed either on a Thermo Quest FTMS using electrospray ionization or JEOL SX-102A using perfluorokerosenes (PFK) as internal standards.

Fermentation Conditions for Production of Apicidins. (1) *Fusarium pallidoroseum* (ATCC 74289, MF6040, isolated

from *Acacia* sp., collected from Santa Rosa National Park, Costa Rica) was inoculated from a frozen stock into YMEJ seed medium (1 mL per 50 mL media in a 250 mL Erlenmeyer flask), consisting of (in g/L): yeast extract, 4; malt extract, 8; glucose, 4; Junlon, 1.5; pH to 7.0. The seed culture was incubated at 25 °C at 220 rpm maintaining 85% rh for 2 days. An aliquot (8−10 mL) of the seed growth was transferred to production medium (MED5) consisting of (in g/L): glucose, 150; fructose, 15; sucrose, 40; N Z amine Type E, 4; urea, 4; K_2HPO_4, 0.5; KCl, 0.25; $MgSO_4 \cdot 7H_2O$, 0.25; $ZnSO_4 \cdot 7H_2O$, 0.9; $CaCO_3$, 8; pH 7.0. The production medium (220 mL) plus inoculum was poured over 675 cm^3 sterile large-particle vermiculite in a 2 L roller bottle. The bottle was shaken vigorously to coat the vermiculite with the liquid medium, and the bottle was incubated horizontally on a Wheaton roller machine (4rpm) at 22 °C for 19 days.

(2) *Fusarium* sp. (ATCC 74322, MF6058, isolated from the roots of *Laguncularia racemosa*, collected from the Osa Peninsula, Costa Rica) was inoculated into seed flasks by aseptically transferring a 1 mL aliquot of a frozen stock culture into a 250 mL Erlenmeyer flask containing 50 mL of KF medium which consists of (in g/L): corn steep powder, 2.5; tomato paste, 40; oat flour, 10; glucose, 10; trace elements solution, 10 mL/L; pH to 6.8. The trace elements solution for the seed medium consisted of (in g/L): $FeSO_4 \cdot 7H_2O$, 1.0; $MnSO_4 \cdot H_2O$, 1.0; $CuCl_2 \cdot 2H_2O$, 0.025; $CaCl_2$, 0.1; H_3BO_3, 0.056; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.019; $ZnSO_4 \cdot 7H_2O$, 0.2. The culture was incubated at 25 °C at 220 rpm for 2−4 days prior to inoculation of the production medium. The production phase was performed on a solid substrate medium in 2 L roller bottles containing approximately 675 cm^3 large-particle vermiculite (measured by volume), with 220 mL of a liquid nutrient solution (NPF2) poured over it. The nutrient solution was formulated as follows (in g/L): glucose, 150; urea, 4; NZ amine Type A, 4; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.25; KCl, 0.25; $ZnSO_4 \cdot 7H_2O$, 0.9; $CaCO_3$, 16.5. The solid and liquid portions of the production medium were combined at the time of inoculation. Each bottle was inoculated with 8−10 mL of the vegetative seed and shaken to coat the vermiculite with the seed growth and nutrient solution. Inoculated bottles were incubated on a Wheaton rolling machine (4 rpm), at 22 °C for 24 days.

Isolation of Apicidins. A 5.6 L fermentation of *F. pallidoroseum* (ATCC 74289) was extracted with 6 L of methyl ethyl ketone. Solvent was evaporated under reduced pressure to give 8.7 g of residue which was triturated with CH_2Cl_2 (400 mL). The filtrate was concentrated to dryness and reconstituted in MeOH−CH_2Cl_2 3:1 (50 mL) and chromatographed over a 1-L Sephadex LH-20 column. Elution with MeOH gave a concentrated solution containing all the apicidins. Concentration and flash chromatography on silica gel using CH_2Cl_2−MeOH 97:3 further removed impurities to give a rich cut weighing 650 mg. The lack of solubility of the apicidins in anything but CH_2Cl_2, chloroform, and the like made the use of reverse-phase HPLC impractical at this point. Rather, it was more convenient to effect a second silica gel fractionation (CH_2Cl_2−EtOAc 3:1) which gave fractions I−IV in order of elution.

Fraction I, containing almost pure apicidin, was triturated with MeOH and filtered and the solid recrystallized from MeOH to afford pure apicidin **1a** (200 mg). Reverse phase HPLC of fraction II on Zorbax RX C-8 (20 × 250 mm) column and elution with 40% aqueous CH_3CN at a flow rate of 8 mL/min gave 5 mg of apicidin C (**1c**). Likewise, reverse phase chromatography of fraction III on the same column and elution with a 100 min gradient of 30−60% aqueous CH_3CN yielded apicidins B (**7a**), D_2 (**1e**), and A (**1b**) in 3, 1, and 1 mg amounts, respectively. Finally, similar chromatography (elution with 35% aqueous CH_3CN) of fraction IV led to the isolation of 5 mg of apicidin D_1 (**1d**). Apicidin D_3 (**1f**) could not be isolated from this batch and was isolated from a larger 10 L fermentation batch in analogous manner. A small fraction eluting between fractions III and IV after the silica gel chromatography contained apicidin D_3 which was chromatographed on reverse phase HPLC following chromatographic conditions of fraction III to give 1.2 mg of apicidin D_3.

Apicidin (1a). For physical and other spectral data see ref 3. HREIMS (*m*/*z*) 623.3738 (calcd for $C_{34}H_{49}N_5O_6$: 623.3687) 2% (M^+), 593.3596 (calcd for $C_{33}H_{47}N_5O_5$: 593.3579) 19%, 394.2095 (calcd for $C_{23}H_{28}N_3O_3$: 394.2131) 5%, 312.2227 (calcd for $C_{16}H_{30}N_3O_3$: 312.2288) 24%, 310.2104 (calcd for $C_{16}H_{28}N_3O_3$: 310.2131) 8%, 283.1438 (calcd for $C_{17}H_{19}N_2O_2$: 283.1447) 45%, 170.0622 (calcd for $C_{11}H_8NO$: 170.0606) 66%, 130.0663 (calcd for C_9H_8N: 130.0657) 61%, 84.0835 (calcd for $C_5H_{10}N$: 84.0814) 100%.

Apicidin A (1b). ^1H NMR (CD_2Cl_2): 0.86 (3H, d, J = 6.4 Hz), 0.94 (3H, t, J = 7.2 Hz), 1.05 (3H, t, J = 7.6 Hz), 1.10−1.70 (13H, m), 1.78 (1H, m), 1.99 (1H, m), 2.01 (1H, m), 2.15 (1H, m), 2.38 (2H, dt, J = 7.2, 1.2 Hz), 2.43 (2H, q, J = 7.6 Hz), 3.10 (1H, dt, J = 13.6, 3.2 Hz), 3.48 (1H, dd, J = 14.4, 6.4 Hz), 3.70 (1H, dd, J = 14.6, 10.0 Hz), 3.99 (1H, m), 4.04 (1H, m), 4.18 (1H, dd, J = 10.6, 8.8, 7.0 Hz), 4.69 (1H, t, J = 10.4 Hz), 5.06 (1H, brd, J = 5.2 Hz), 6.36 (1H, d, J = 5.6 Hz), 6.41 (1H, d, J = 10.4 Hz), 6.99 (1H, d, J = 10.4 Hz), 7.10 (1H, brs), 7.12 (1H, ddd, J = 8.0, 6.8, 0.8 Hz), 7.18 (1H, ddd, J = 8.4, 7.2, 1.2 Hz), 7.39 (1H, dt, J = 8.4, 1.2 Hz), 7.60 (1H, dd, J = 7.6, 0.4 Hz), 8.67 (1H, brs); HREIMS (*m*/*z*) 593.3596 (calcd for $C_{33}H_{47}N_5O_5$: 593.3579), M^+, 100%, 394.2119 (calcd for $C_{23}H_{28}N_3O_3$: 394.2131) 15%, 312.2265 (calcd for $C_{16}H_{30}N_3O_3$: 312.2288) 48%, 310.2097 (calcd for $C_{16}H_{28}N_3O_3$: 310.2131) 14%, 283.1448 (calcd for $C_{17}H_{19}N_2O_2$: 283.1447) 89%, 170.0621 (calcd for $C_{11}H_8NO$: 170.0606) 87%, 130.0663 (calcd for C_9H_8N: 130.0657) 80%, 84.0835 (calcd for $C_5H_{10}N$: 84.0814) 87%.

Apicidin B (7a). For physical and other spectral data see ref 16. HREIMS (*m*/*z*) 609.3525 (calcd for $C_{33}H_{47}N_5O_6$: 609.3529), M^+, 9%, 579.3404 (calcd for $C_{32}H_{45}N_5O_5$: 579.3423) 100%, 380.1984 (calcd for $C_{22}H_{26}N_3O_3$: 380.1976) 5%, 298.2097 (calcd for $C_{15}H_{28}N_3O_3$: 298.2132) 25%, 296.1983 (calcd for $C_{15}H_{26}N_3O_3$: 296.1976) 5%, 283.1449 (calcd for $C_{17}H_{19}N_2O_2$: 283.1447) 39%, 170.0616 (calcd for $C_{11}H_8NO$: 170.0606) 68%, 130.0659 (calcd for C_9H_8N: 130.0657) 47%, 70.0674 (calcd for C_4H_8N: 70.0657) 29%.

Apicidin C (1c). For physical and other spectral data see ref 16. HREIMS (*m*/*z*)) 609.3512 (calcd for $C_{33}H_{47}N_5O_6$: 609.3529), M^+, 10%, 579.3404 (calcd for $C_{32}H_{45}N_5O_5$: 579.3423) 79%, 380.1961 (calcd for $C_{22}H_{26}N_3O_3$: 380.1976) 15%, 312.2261 (calcd for $C_{16}H_{30}N_3O_3$: 312.2288) 47%, 310.2083 (calcd for $C_{16}H_{28}N_3O_3$: 310.2131) 14%, 269.1262 (calcd for $C_{16}H_{17}N_2O_2$: 269.1291) 71%, 170.0605 (calcd for $C_{11}H_8NO$: 170.0606) 86%, 130.0655 (calcd for C_9H_8N: 130.0657) 88%, 84.0843 (calcd for $C_5H_{10}N$: 84.0814) 100%.

Apicidin D_1 (1d). Amorphous powder, $[\alpha]^{22}_D$ −72.6° (*c*, 0.2, $CHCl_3$), IR ν_{max} (ZnSe) 3332, 2900, 1690, 1658, 1622, 1532, 1455, 1271, 1244, 749 cm^{-1}. For ^1H and ^{13}C NMR see Table 1. HREIMS (*m*/*z*) 639.3575 (calcd for $C_{34}H_{49}N_5O_7$: 639.3633) 6% (M^+), 607.3387 (calcd for $C_{33}H_{45}N_5O_6$: 607.3371) 47%, 394.2151 (calcd for $C_{23}H_{28}N_3O_3$: 394.2131) 11%, 326.2055 (calcd for $C_{16}H_{28}N_3O_4$: 326.2081) 24%, 283.1446 (calcd for $C_{17}H_{19}N_2O_2$: 283.1447) 88%, 170.0581 (calcd for $C_{11}H_8NO$: 170.0606) 100%, 84.0830 (calcd for $C_5H_{10}N$: 84.0814) 77%.

Apicidin D_2 (1e). Amorphous powder, $[\alpha]^{22}_D$ −68.5° (*c*, 0.26, $CHCl_3$), IR ν_{max} (ZnSe) 3350 (br), 2954, 1667, 1622, 1527, 1442, 1271, 1244, 749 cm^{-1}. For ^1H and ^{13}C NMR see Table 1. HREIMS (*m*/*z*) 625.3802 (calcd for $C_{34}H_{51}N_5O_6$: 625.3840) 8% (M^+), 607.3730 (calcd for $C_{34}H_{49}N_5O_5$: 607.3735) 2%, 593.3539 (calcd for $C_{33}H_{47}N_5O_5$: 593.3578) 30%, 394.2124 (calcd for $C_{23}H_{28}N_3O_3$: 394.2131) 16%, 314.2398 (calcd for $C_{16}H_{32}N_3O_3$: 314.2444) 12%, 312.2264 (calcd for $C_{16}H_{30}N_3O_3$: 312.2288) 6%, 283.1444 (calcd for $C_{17}H_{19}N_2O_2$: 283.1447) 90%, 170.0605 (calcd for $C_{11}H_8NO$: 170.0606) 92%, 130.0657 (calcd for C_9H_8N: 130.0657) 83%, 84.0841 (calcd for $C_5H_{10}N$: 84.0814) 100%.

Apicidin D_3 (1f). Amorphous powder, $[\alpha]^{22}_D$ − 60.4° (*c*, 0.26, $CHCl_3$), IR ν_{max} (ZnSe) 3332 (br), 2945, 1685, 1626, 1523, 1460, 1415, 1280, 1244, 744 cm^{-1}. For ^1H and ^{13}C NMR see Table 1. HREIMS (*m*/*z*) 625.3835 (calcd for $C_{34}H_{51}N_5O_6$: 625.3840) 5% (M^+), 607.3730 (calcd for $C_{34}H_{49}N_5O_5$: 607.3735) 2%, 577.3606 (calcd for $C_{33}H_{47}N_5O_4$: 577.3629) 20%, 394.2127 (calcd for $C_{23}H_{28}N_3O_3$: 394.2131) 14%, 314.2449 (calcd for $C_{16}H_{32}N_3O_3$: 314.2444) 47%, 312.2294 (calcd for $C_{16}H_{30}N_3O_3$: 312.2288) 20%, 283.1426 (calcd for $C_{17}H_{19}N_2O_2$: 283.1447) 100%, 170.0609

(calcd for $C_{11}H_8NO$: 170.0606) 94%, 130.0657 (calcd for C_9H_8N: 130.0657) 62%, 84.0842 (calcd for $C_5H_{10}N$: 84.0814) 80%.

Apicidin A (1b) from Apicidin (1a). To a solution of apicidin (2.8 mg) in a 2 mL (1:1) mixture of MeOH and EtOAc was added 10% palladium over charcoal (5 mg), and the mixture was evacuated to remove dissolved air. This mixture was flushed with hydrogen, and the degassing and flushing cycle was repeated three times. Finally the hydrogen was connected to the flask, and the mixture was stirred for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated under reduced pressure to give 2.6 mg of *N*-desmethoxyapicidin (apicidin A, **1b**) as a colorless powder which was identical to the natural apicidin A by NMR, EIMS and HPLC.

Desmethoxyapicidin B (7b). A similar hydrogenolysis of apicidin B (0.8 mg) gave 0.7 mg of *N*-desmethoxyapicidin B as a colorless powder, ^{1}H NMR (CD_2Cl_2): 0.86 (3H, d, J = 6.5 Hz), 0.91 (3H, t, J = 7.5 Hz), 1.03 (3H, t, J = 7.0 Hz), 1.10−1.70 (8H, m), 1.78 (3H, m), 1.93 (1H, m), 1.96 (1H, m), 2.22 (1H, m), 2.32 (1H, m), 2.36 (2H, dt, J = 7.5, 3.0 Hz), 2.42 (2H, q, J = 7.0 Hz), 3.49 (1H, dd, J = 10, 7.5 Hz, Pro-δH), 3.53 (1H, dd, J = 14, 7 Hz, Trp-βH), 3.76 (1H, dd, J = 14.5, 9.5 Hz, Trp-βH), 3.81 (1H, dt, J = 9.8, 4.5 Hz, Pro-δH), 3.98 (1H, dt, J = 10, 7 Hz, Trp-αH), 4.14 (1H, ddd, J = 10, 8.5, 6.5 Hz, Aoda-αH), 4.50 (1H, t, J = 10.5 Hz, Ile-αH), 4.72 (1H, dd, J = 7.5, 1.5 Hz, Pro-αH), 6.30 (1H, d, J = 6 Hz, Trp-NH), 7.06 (1H, d, J = 10.5 Hz, NH), 7.10 (1H, dd, J = 8, 1 Hz), 7.14 (1H, d, J = 10.5 Hz, NH), 7.16 (1H, dt, J = 8, 0.5 Hz), 7.36 (1H, d, J = 8.5 Hz), 7.58 (1H, d, J = 8 Hz), 8.55 (1H, brs, NH); ESI (*m*/*z*): 580 (M + H).

(8*R*,*S*)-Hydroxyapicidin (8). To a solution of apicidin (20 mg, 0.032 mmol) in a mixture of MeOH (1 mL) and CH_2Cl_2 (1 mL) was added $NaBH_4$ (3 mg, 0.08 mmol), and the solution was stirred at ambient temperature for 30 min and quenched with acetone. The solution was diluted with EtOAc (20 mL), washed with H_2O (10 mL), dried, and evaporated under reduced pressure and the residue chromatographed on a small column of silica gel eluted with 2.5% MeOH−CH_2Cl_2 to give 17 mg of 1:1 inseparable diastereomeric mixture of C-8 hydroxy apicidin **8** as a colorless powder. The reaction was easily scaled up to 350 mg scale. ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, d, J = 6.4 Hz), 0.91, 0.92 (3H, t, J = 7.2 Hz), 0.93 (3H, t, J = 7.6 Hz), 1.10−1.61 (15H, m), 1.72 (2H, m), 1.80−2.2 (4H, m), 2.98 (1H, dt, J = 12.8, 2.0 Hz), 3.46 (1H, m), 3.48 (1H, t, J = 7.6 Hz), 3.65 (1H, dd, J = 14.8, 10 Hz), 4.03 (3H, s), 4.04 (1H, m), 4.25 (1H, q, J = 8 Hz), 4.70 (1H, t, J = 10.4 Hz), 5.06 (1H, brd, 4.4 Hz), 6.48 (1H, d, J = 10 Hz), 6.90, 6.91 (1H, d, J = 6.8 Hz), 7.10 (1H, dt, J = 8,1.2 Hz), 7.11 (1H, d, J = 10.8 Hz), 7.17 (1H, s), 7.24 (1H, dt, J = 8, 0.8 Hz), 7.40 (1H, d, J = 8 Hz), 7.57 (1H, d, J = 8 Hz). ESIMS (*m*/*z*): 626 (M + H).

(8*R*,*S*)-Acetoxyapicidin (9). To a solution of the diastereomeric mixture of hydroxyapicidin (20 mg) in C_5H_5N (0.5 mL) was added Ac_2O (0.4 mL), and the solution was stirred at room-temperature overnight. MeOH was added to destroy unreacted Ac_2O, and then all volatiles were removed under reduced pressure, dissolved in EtOAc (60 mL), washed twice with 30 mL each with 10% aqueous citric acid, H_2O, and 10% aqueous $NaHCO_3$, followed by H_2O, dried, evaporated under reduced pressure, and chromatographed on preparative TLC (hexane−acetone, 1:1). The band was extracted with acetone to give 17 mg of diastereomeric acetate (**9**) as colorless powder. ^{1}H NMR ($CDCl_3$): 0.85 (3H, d, J = 6 Hz), 0.86 (3H, t, J = 7.6 Hz), 0.92 (3H, t, J = 7.6 Hz), 1.10−1.61 (15H, m), 1.75 (3H, m), 1.98, 2.05 (3H, s, $COCH_3$), 3.04 (1H, brt, J = 13.2 Hz), 3.47 (1H, dt, J = 14.4, 6.4 Hz), 3.81 (1H, ddd, J = 14.8, 10.8, 4.4 Hz), 3.95 (1H, m), 4.02 (3H, s), 4.17 (1H, m), 4.72 (1H, t, J = 10 Hz), 4.78 (1H, dd, J = 12.8, 5.6 Hz), 5.06 (1H, brd, 6.0 Hz), 6.41 (1H, dd, J = 11.2, 6.0 Hz), 6.45 (1H, d, J = 10.4 Hz), 7.08 (1H, t, J = 7.6 Hz), 7.11 (1H, s), 7.23 (1H, t, J = 7.2 Hz), 7.39 (1H, d, J = 8 Hz), 7.56 (1H, d, J = 8 Hz). HREIMS (*m*/*z*): 667.3903 (calcd for $C_{36}H_{53}N_5O_7$: 667.3945).

8-Deoxyapicidin A (12). Methanesulfonyl chloride (0.005 mL) was added to a solution of diastereomeric alcohol (6.2 mg), DIPEA (0.020 mL), and DMAP (10 mg) in 1 mL of CH_2Cl_2. The solution was stirred at room-temperature overnight, which produced a less polar product (TLC, SiO_2, CH_2Cl_2−MeOH, 96:4). EtOAc (20 mL) was added, and the solution was washed with 10% aqueous citric acid, H_2O, 10% aqueous $NaHCO_3$, and H_2O. Evaporation of EtOAc under reduced pressure gave mesylate (**10**) which was dissolved in 1 mL of toluene and heated at reflux overnight with DBU (0.020 mL). The reaction produced less polar products which gave a chromatographically inseparable 1:1 mixture (^{1}H NMR) of Δ^7 and Δ^9 dehydro compounds (**11**, [ESIMS (*m*/*z*) 608 (M + H]) after the same acid−base workup. These were dissolved in a mixture of EtOAc (1 mL) and MeOH (0.25 mL) and were hydrogenated as described earlier. Purification on a Pasteur pipet filled with silica gel and elution with 5% MeOH−CH_2Cl_2 gave 1.8 mg of 8-deoxyapicidin A (**12**) as a colorless powder. ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, d, J = 6.4 Hz), 0.89 (3H, t, J = 6.8 Hz), 0.93 (3H, t, J = 7.2 Hz), 1.1−1.85 (22H, m), 1.98−2.2 (3H, m), 3.06 (1H, brt, J = 12.8 Hz), 3.50 (1H, dd, J = 12.8, 5.6 Hz), 3.84 (1H, dd, J = 14.8, 10.4 Hz), 4.10 (2H, m), 4.14 (1H, m), 4.72 (1H, t, J = 10.4 Hz), 5.05 (1H, brd, J = 5.6 Hz),6.23 (1H, d, J = 6.0 Hz), 6.42, 6.44 (1H, d, J = 10.4 Hz, NH), 7.04 (1H, d, J = 2.0 Hz), 7.11 (1H, dt, J = 8.0, 0.8 Hz), 7.17 (1H, d, J = 10.8 Hz, NH), 7.20 (1H, dt, J = 8.0, 0.8 Hz), 7.36 (1H, d, J = 8 Hz), 7.60 (1H, d, J = 7.6 Hz), 8.00 (1H, brs, NH). ESIMS (*m*/*z*) 580 (M + H).

Apicidin 8-*O*-Benzyloxime (13). A solution of apicidin (4 mg), *O*-benzylhydroxylamine hydrochloride (20 mg), DIPEA (0.2 mL), and DMAP (10 mg) in CH_2Cl_2 (1 mL) was stirred at room-temperature overnight and then heated at 60 °C for 10 h. EtOAc (50 mL) was added, and the solution was washed with 10% aqueous citric acid and H_2O. EtOAc extract was dried, evaporated under reduced pressure, and chromatographed by preparative silica gel TLC (CH_2Cl_2−MeOH, 97:3). The band was eluted with acetone to give 1.5 mg of a 1:1 mixture of the geometrical isomers of oxime **13** as colorless powder. ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, d, J = 6.4 Hz), 0.93 (3H, t, J = 7.6 Hz), 1.03, 1.07 (3H, t, J = 7.2 Hz), 1.2−1.85 (16H, m), 1.90−2.10 (3H, m), 2.17 (2H, q, J = 7.2 Hz), 2.25−2.35 (2H, m), 3.05 (1H, brt, J = 13.2 Hz), 3.45 (1H, m), 3.81 (1H, m), 3.92 (1H, m), 3.99, 4.00 (3H, s), 4.15 (1H, m), 4.73 (1H, t, J = 10.4 Hz), 5.04 (1H, brd, J = 5.6 Hz), 5.04, 5.06 (2H, s), 6.22 (1/2H, d, J = 6.0 Hz, NH), 6.41 (1/2H, d, J = 5.6 Hz, NH), 6.43, 6.46 (1H, d, J = 9.6 Hz, NH), 7.16 (2H, m), 7.17 (1H, d, J = 10.8 Hz, NH), 7.23 (1H, t, J = 8 Hz), 7.27 (1H, m), 7.32−7.35 (4H, m), 7.38 (1H, d, J = 8 Hz), 7.55 (1H, d, J = 8 Hz), ESIMS (*m*/*z*) 729 (M + H).

Acyclic Apicidin Ethyl Ester (14). To a solution of apicidin (623 mg) in a mixture of EtOH (12 mL), CH_2Cl_2 (12 mL), and THF (8 mL) was added PTSA·H_2O (400 mg), and the solution was stirred at room-temperature overnight followed by heating at 50 °C for 4 h. Solvents were removed under reduced pressure, 100 mL of EtOAc was added, and the solution was washed with 10% aqueous $NaHCO_3$ (2 × 20 mL) and H_2O (20 mL). EtOAc extract was dried evaporated under reduced pressure to give 600 mg (90% yield) of **14** as chromatographically homogeneous powder, which was crystallized from acetone−hexane as small colorless needles. mp 133−35 °C, ^{1}H NMR ($CDCl_3$) δ: 0.78 (3H, d, J = 6.8 Hz), 0.84 (3H, t, J = 7.6 Hz), 1.04 (3H, t, J = 6.8 Hz), 1.24 (3H, t, J = 6.8 Hz), 1.04, 1.2−1.9 (17H, m), 2.37 (2H, t, J = 7.2 Hz), 2.40 (2H, q, J = 7.2 Hz), 2.69 (1H, m), 3.05 (1H, m), 3.11 (1H, dd, J = 14.8, 7.2 Hz), 3.26 (1H, m), 3.33 (1H, dd, 14.4, 6 Hz), 4.06 (3H, s), 4.12 (2H, dq, 6.8, 1.2 Hz), 4.23 (1H, q, 8 Hz), 4.39 (1H, dd, J = 8.4, 5.2 Hz), 4.72 (1H, dd, 13.6, 7.2 Hz), 6.50 (1H, br), 7.14 (1H, t, J = 7.2 Hz), 7.23 (1H, s), 7.23 (1H, t, J = 7.2 Hz), 7.38 (1H, d, J = 8 Hz), 7.66 (1H, d, J = 8.4 Hz); ESIMS (*m*/*z*): 670 (M + H).

Acyclic Apicidin Methyl Ester (15). Similar reaction in methanol afforded methyl ester **15**. ^{1}H NMR ($CDCl_3$) δ: Pip 3.23 (1H, m, H-2), 1.06, 1.74 (2H, m, H_2-3), 1.29, 1.32 (2H, m, H_2-4), 1.38, 1.58 (2H, m, H_2-5), 2.69 (1H, dt, J = 11.5, 0.5 Hz, H-6) 3.05 (1H, brd, J = 10 Hz, H-6); Aoda: 4.15 (1H, brdt, J = 8, 6 Hz, H-2), 1.60, 1.77 (2H, m, H_2-3), 1.25−1.28 (4H, m, H_2-4, 5), 1.50 (2H, m, H_2-6), 2.38 (2H, t, J = 7.5 Hz, H_2-7), 2.40 (2H, q, J = 7.5 Hz, H_2-9), 1.02 (3H, t, J = 7 Hz, H_3-10),

7.24 (1H, d, J = 7.5 Hz, NH); Trp: 4.69 (1H, dt, J = 7.7, 6.5 Hz, H-2), 3.11 (1H, dd, J = 14.5, 6.5 Hz, H-3), 3.33 (1H, dd, J = 15, 6 Hz, H-3), 7.23 (1H, s, H-2′), 7.65 (1H, d, J = 8 Hz, H-5′), 7.13 (1H, dt, J = 8, 1 Hz, H-6′), 7.25 (1H, dt, J = 8, 1 Hz, H-7′), 7.42 (1H, d, J = 8 Hz, H-8′), 4.06 (3H, s, OCH_3), 6.63 (1H, brd, J = 9 Hz, NH); Ile: 4.39 (1H, dd, J = 8.5, 6 Hz, H-2), 1.77 (1H, m, H-3), 1.09, 1.40 (2H, m, H_2-4), 0.84 (3H, t, J = 7.5 Hz, H_3-5), 0.79 (3H, d, J = 7 Hz, H_3-6), 3.67 (3H, s, CO_2CH_3), 6.93 (1H, br, NH). ^{13}C NMR (CD_2Cl_2) δ: Pip: 174.3, 60.0, 29.5, 24.0, 25.5, 45.9; Aoda: 171.6, 54.2, 31.7, 26.0, 29.1, 23.8, 42.4, 211.7, 36.1, 8.0; Trp: 171.3, 54.1, 27.7, 122.7, 106.6, 124.3, 119.4, 120.2, 122.8, 108.7, 132.7, 66.2; Ile: 172.4, 57.2, 37.7, 25.8, 11.5, 15.4, 52.5. ESIMS (m/z): 656 (M + H).

Acyclic Apicidin (16). To a solution of **14** (550 mg, 0.82 mmol) in THF (6 mL) was added a 2 mL water solution of LiOH (66 mg, 2.8 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was quenched by addition of 6 N HCl (2 mL) and diluted with 100 mL of EtOAc, and the extract was washed with a saturated solution of NaCl. Drying followed by evaporation of the solvent under reduced pressure and drying in a vacuum desiccator over P_2O_5 gave clean acid **16** (520 mg, 98%) as a powder. A small aliquot was purified by reversed phase HPLC using a 25 min 10 to 90% aqueous CH_3CN containing 0.1% TFA on a Zorbax RX C-8 (22 × 250 mm) column at a flow rate of 8 mL/min. Lyophilization of fractions gave **16** as an colorless powder. ^{1}H NMR ($CD_3OD-CDCl_3$, 1:1) δ: 0.88 (3H, d, J = 8 Hz), 0.89 (3H, t, J = 8.5 Hz), 1.02 (3H, t, J = 7.5 Hz), 1.2−2.2 (17H, m), 2.40 (2H, t, J = 7.0 Hz), 2.42 (2H, q, J = 7.0 Hz), 2.95 (1H, dt, J = 13, 3 Hz), 3.10 (1H, dd, J = 14.5, 7.0 Hz), 3.24 (1H, dd, J = 15, 6.5 Hz), 3.39 (1H, brd, J = 13 Hz), 3.71 (1H, dd, J = 12, 3 Hz), 4.04 (3H, s), 4.20 (1H, dd, J = 8.5, 5 Hz), 4.35 (1H, d, J = 5.0 Hz), 4.72 (1H, m), 7.06 (1H, t, J = 7.2 Hz), 7.19 (1H, t, J = 7.2 Hz), 7.26 (1H, s), 7.35 (1H, d, J = 8 Hz), 7.59(1H, d, J = 8.4 Hz); ESIMS (m/z): 641 (M + H).

(8*R*)- and (8*S*)-Apicidin (*R*)-Methoxymandelate (17 and 18). Similar reaction of diastereomeric alcohol with (*R*)-methoxymandelic acid and chromatography afforded of (8*R*,*R*)-mandelate **17** (t_R = 23.04 min, LC-A) and (8*S*,*R*)-mandelate **18** (t_R = 23.90 min, LC-A). **17**: ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, J = 7.6 Hz), 0.87 (3H, d, J = 6.4 Hz), 0.92 (3H, t, J = 7.6 Hz), 1.02 (2H, m), 1.16 (1H, m), 1.3−1.42 (6H, m), 1.55−1.70 (7H, m), 1.80 (1H, brd, J = 8 Hz), 1.90−2.2 (3H, m), 3.06 (1H, dt, J = 12, 1 Hz), 3.40 (3H, s), 3.48 (2H, dd, J = 12, 6.8 Hz), 3.78 (1H, dd, J = 12.4, 10 Hz), 3.94 (1H, m), 4.00 (3H, s), 4.04 (1H, brd, J = 8 Hz), 4.12 (1H, q, J = 7.6 Hz), 4.73 (1H, t, J = 10 Hz), 4.73 (1H, s), 4.84 (1H, pentet, J = 6.8 Hz), 5.07 (1H, brd, 4.4 Hz), 6.21 (1H, br, NH), 6.41 (1H, d, J = 10.4 Hz), 7.09 (1H, s), 7.09 (1H, dt, J = 8, 0.8 Hz), 7.18 (1H, d, J = 10 Hz), 7.23 (1H, dt, J = 7.2, 1.2 Hz), 7.32 (3H, m), 7.39 (1H, d, J = 8.4 Hz), 7.42 (2H, dd, J = 8.4, 2.4 Hz), 7.55 (1H, d, J = 8 Hz). HREIMS (m/z) 773.4324 (calcd for $C_{43}H_{59}N_5O_8$: 773.4365). **18**: ^{1}H NMR ($CDCl_3$) δ 0.58 (3H, t, J = 7.6 Hz), 0.87 (3H, d, J = 6.4 Hz), 0.92 (3H, t, J = 7.2 Hz), 1.16 (5H, m), 1.3−1.42 (6H, m), 1.50−1.65 (5H, m), 1.81 (1H, brd, J = 8 Hz), 1.90−2.2 (3H, m), 3.06 (1H, dt, J = 13.2, 2.4 Hz), 3.41 (3H, s), 3.49 (2H, dd, J = 12.4, 7.6 Hz), 3.80 (1H, dd, J = 14.4, 10 Hz), 3.96 (1H, dt, J = 9.6, 6.8 Hz), 4.02 (3H, s), 4.04 (1H, brd, J = 8 Hz), 4.15 (1H, q, J = 7.6 Hz), 4.73 (1H, t, J = 10.4 Hz), 4.76 (1H, s), 4.83 (1H, ddd, J = 12.4, 7.2, 4.8 Hz), 5.06 (1H, brd, 6.0 Hz), 6.30 (1H, brd, J = 6.4 Hz, NH), 6.44 (1H, d, J = 10 Hz), 7.10 (1H, dt, J = 8, 0.8 Hz), 7.11, (1H, s), 7.19 (1H, d, J = 10 Hz), 7.24 (1H, dt, J = 8, 0.8 Hz), 7.32 (3H, m), 7.40 (1H, d, J = 8 Hz), 7.44 (2H, dd, J = 8, 2 Hz), 7.56 (1H, d, J = 8 Hz). HREIMS (m/z) 773.4406 (calcd for $C_{43}H_{59}N_5O_8$: 773.4365).

(8*S*)-Apicidin D_2 (*R*)-Methoxymandelate (18). Apicidin D_2 (2 mg) was similarly reacted with (*R*)-methoxymandelic acid, and after workup it was purified by reverse phase HPLC (Zorbax RX C-8, 4.6 × 250 mm, 65% aqueous CH_3CN, 1 mL/min). The product eluting at 23.9 min was lyophilized to give 1.4 mg of colorless mandelate which was identical (HPLC, NMR, MS) to the (8*S*,*R*)-methoxymandelate (**18**) described earlier.

(8*R*)- and (8*S*)-Apicidin (*S*)-Methoxymandelate (19 and 20). Oxalyl chloride (0.08 mL, 0.16 mmol) was added to a cooled (0 °C) solution of (*S*)-methoxymandelic acid (26.5 mg, 0.16 mmol) and DMF (0.019 mL, 0.24 mmol) in 1 mL of CH_3CN under N_2 and stirred for 20 min. A solution of diastereomeric mixture of hydroxyapicidin (50 mg, 0.08 mmol) in 1.5 mL of CH_2Cl_2 was added, followed by addition of pyridine (0.026 mL, 0.32 mmol). The solution was stirred at 0 °C for 1 h followed by overnight at room temperature. The reaction was quenched by addition of ice and then poured on to EtOAc (60 mL) and which was washed twice each with 30 mL each of 10% aqueous citric acid, H_2O, 10% aqueous $NaHCO_3$, followed by H_2O, dried, evaporated under reduced pressure, and chromatographed by preparative TLC to give 40 mg of diastereomeric mixture of acetate. Twenty milligrams of the mixture of diastereomers was dissolved in 0.35 mL 3:1 mixture of MeOH−DMSO and was separated by reverse phase HPLC on Zorbax RX C-8 (9.4 × 250 mm) at 50 °C eluting with 50% aqueous CH_3CN at a flow rate of 2 mL/min to afford 2 mg each of pure 8*S* *S*-mandelate **19** (t_R = 22.78 min, Zorbax RX C-8, 4.6 × 250 mm, 65% aqueous CH_3CN, 1 mL/min, LC-A) and (8*R*,*S*)-mandelate **20** (t_R = 24.08 min, LC-A). **19**: ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, t, J = 7.6 Hz), 0.87 (3H, d, J = 6.4 Hz), 0.93 (3H, t, J = 7.2 Hz), 1.00 (2H, m), 1.16 (1H, m), 1.3−1.42 (6H, m), 1.50−1.65 (7H, m), 1.82 (1H, brd, J = 8 Hz), 1.90−2.2 (3H, m), 3.06 (1H, dt, J = 12.8, 2.8 Hz), 3.41 (3H, s), 3.48 (2H, dd, J = 10, 4.4 Hz), 3.80 (1H, dd, J = 14.4, 10 Hz), 3.95 (1H, m), 4.01 (3H, s), 4.04 (1H, brd, J = 8 Hz), 4.10 (1H, q, J = 8.4 Hz), 4.73 (1H, t, J = 10.4 Hz), 4.74 (1H, s), 4.83 (1H, pentet, J = 6.4 Hz), 5.06 (1H, brd, 4.4 Hz), 6.21 (1H, br, NH), 6.41 (1H, d, J = 10.4 Hz), 7.09 (1H, s), 7.09 (1H, dt, J = 7.4, 0.8 Hz), 7.16 (1H, d, J = 10 Hz), 7.23 (1H, dt, J = 7.6, 1.2 Hz), 7.32 (3H, m), 7.39 (1H, d, J = 8 Hz), 7.44 (2H, dd, J = 8, 2 Hz), 7.55 (1H, d, J = 8 Hz). HREIMS (m/z) 773.4293 (calcd for $C_{43}H_{59}N_5O_8$: 773.4365). **20** ^{1}H NMR ($CDCl_3$) δ 0.59 (3H, t, J = 7.2 Hz), 0.87 (3H, d, J = 6.4 Hz), 0.92 (3H, t, J = 7.6 Hz), 1.16 (5H, m), 1.3−1.42 (6H, m), 1.50−1.65 (5H, m), 1.81 (1H, brd, J = 8 Hz), 1.90−2.2 (3H, m), 3.06 (1H, dt, J = 12.8, 1.2 Hz), 3.41 (3H, s), 3.49 (2H, dd, J = 12.4, 7.2 Hz), 3.80 (1H, dd, J = 14.4, 10 Hz), 3.96 (1H, dt, J = 10.4, 6.4 Hz), 4.02 (3H, s), 4.03 (1H, brd, J = 8 Hz), 4.16 (1H, q, J = 7.6 Hz), 4.73 (1H, t, J = 10 Hz), 4.74 (1H, s), 4.83 (1H, ddd, J = 12.4, 7.2, 5.2 Hz), 5.06 (1H, brd, 4.4 Hz), 6.27 (1H, brd, J = 6 Hz, NH), 6.44 (1H, d, J = 10.4 Hz), 7.09 (1H, dt, J = 7.2, 1.2 Hz), 7.10, (1H, s), 7.19 (1H, d, J = 10.4 Hz), 7.23 (1H, dt, J = 8, 0.8 Hz), 7.32 (3H, m), 7.39 (1H, d, J = 8.4 Hz), 7.44 (2H, dd, J = 8, 1.6 Hz), 7.56 (1H, d, J = 7.6 Hz). HREIMS (m/z) 773.4372 (calcd for $C_{43}H_{59}N_5O_8$: 773.4365).

Dehydroapicidin D_3 (21). To a cooled (−60 °C) solution of DMSO (0.073 mL) in CH_2Cl_2 (1 mL) was added oxalyl chloride (0.025 mL), and the solution was stirred for 10 min. Apicidin D_3 (2 mg) was added, and the solution was stirred for 30 min followed by addition of TEA, and after stirring at −60 °C for 20 min, the reaction was allowed to warm to room temperature. The reaction mixture was quenched with ice and poured onto EtOAc (5 mL). The EtOAc layer was washed with 5 mL each of 10% aqueous citric acid and water, dried (Na_2SO_4), evaporated under vacuo, and chromatographed on an analytical Zorbax RX C-8 column (4.6 × 250 mm). Elution with 50% aqueous CH_3CN gave 1.5 mg of the desired oxidized product **21** as a gum. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 7.6 Hz), 0.91 (3H, d, J = 6.8 Hz), 1.1−1.3 (8H, m), 1.4−1.7 (6H, m), 1.80 (1H, brd, J = 9.2 Hz), 1.94 (2H, m), 2.05 (1H, m), 2.10 (3H, s), 2.37 (2H, t, J = 7.6 Hz), 2.74 (1H, dd, J = 14.8, 10 Hz), 2.98 (1H, d, J = 15.2 Hz), 3.28 (1H, brt, J = 13.6 Hz), 3.83 (1H, m), 3.88 (3H, s), 4.33 (1H, q, J = 6.8 Hz), 4.80 (1H, t, J = 9.6 Hz), 4.83 (1H, t, J = 9.6 Hz), 5.10 (1H, brd, J = 5.6 Hz), 6.06 (1H, d, J = 10 Hz), 6.26 (1H, d, J = 6.4 Hz), 6.27 (1H, brs), 7.08 (1H, d, J = 8 Hz), 7.16 (1H, dt, J = 7.2, 0.8 Hz), 7.26 (1H, s), 7.32 (1H, d, J = 7.6 Hz), 7.36 (1H, dt, J = 7.6, 1.2 Hz). HREIMS (m/z) 623.3604 (calcd for $C_{34}H_{49}N_5O_5$: 623.3682).

Acknowledgment. The authors thank Chris Cannova, Tami Crumley, Paula Dulski, and Marta Arocho for technical help and Instituto Nacional de Biodiver-

sidad, Santo Domingo de Heredia, Costa Rica, for assistance in collecting the plant materials for isolation of fungi.

Note Added in Proof. Synthesis of apicidin (Kuriyama, W.; Kitahara, T. *Heterocycle* **2001**, *55*, 1 and 1835) and apicidin A (Mou, L.; Singh, G. *Tetrahedron Lett.* **2001**, *42*, 6603) was reported after submission of the manuscript. The tetrapeptide cyclization was accomplished between Pip and Aoda in both of these syntheses.

JO016088W