Cimilophytine, a New Bisindole Alkaloid from *Haplophyton cimicidum* (Apocynaceae)

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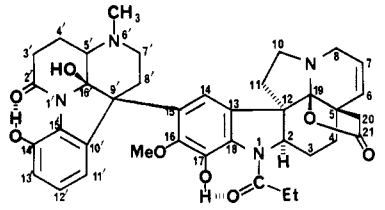
Cimilophytine, a new bisindole alkaloid of the aspidosperma–canthinone structural class, isolated from *Haplophyton cimicidum* (Apocynaceae), has been assigned structure 1.

The structures of a number of indole alkaloids from *Haplophyton cimicidum* have been reported[1-4] from our laboratory. Two of these alkaloids, cimiciphytine (2) and norcimiciphytine (3) were isolated by subjecting the al-



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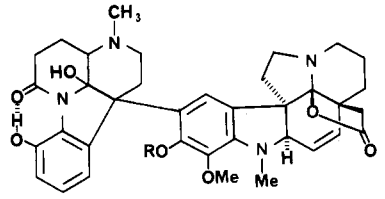
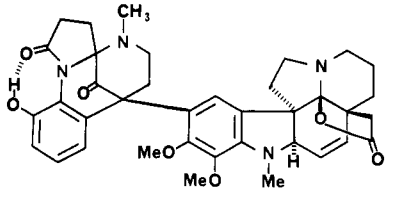


2 R=CH_3
3 R=H



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kali-soluble base residues from the isolation of haplophytine (4), the major alkaloid of *H. cimicidum*, to pH-gradient countercurrent distribution.[4] We now report the structure of another new bisindole alkaloid, cimilophytine (1), which we have isolated from the same alkali-soluble base residues.

Cimilophytine (1), $C_{38}H_{42}N_4O_8$ (by high-resolution mass spectrometry), crystallized as small plates from ethanol [mp 325 °C dec, $[\alpha]^{20}$ -84.9° (EtOH)]. The mass spectrum of 1 exhibited a weak molecular ion at m/e 682 (3%) and significant fragment ions at m/e 664 (79), 623 (100), 605 (20), 580 (14), 411 (21), 351 (27), 255 (50), 172 (22), 170 (24), 159 (20), 158 (20), 134 (16), and 130 (20).

The NMR spectrum of 1 ($CDCl_3$) showed one *C*-methyl of an *N*-propionyl function at δ 1.33 (t, J = 7 Hz, 3 H) coupled to a two-proton quartet at δ 2.64. It also showed one aliphatic *N*-methyl at δ 2.39 (s, 3 H), a shielded methoxyl at δ 3.24 (s, 3 H), two vinyl protons at δ 5.66 (s, 2 H), and four aromatic protons δ 6.19 (dd, J = 7, 1 Hz, 1 H), 6.82 (dd, J = 8, 1 Hz, 1 H), 6.97 (dt, J = 8, 1 Hz, 1 H), and 7.25 (s, 1 H). Three D_2O-exchangeable protons were observed at δ 7.08, 10.16, and 11.49.

The KBr infrared spectrum of 1 showed hydroxyl absorption at 3425 cm^{-1} and carbonyl absorptions at 1751 (lactone C=O) and 1621 cm^{-1} (amide C=O). The UV absorption spectrum of cimilophytine [λ_{max}^{EtOH} 228 (32 800), 266 (15 900), and 300 (4000) nm] indicated the presence of a dihydroindole system.[5] The band at 266 nm underwent a bathochromic shift to 315 nm (13 800) upon addition of alkali, indicating the presence of a phenolic function.

The assignment of structure 1 to cimilophytine was based on its spectral and chemical properties. The mass spectral fragmentation of 1, studied with the aid of high-resolution mass spectrometry, was particularly useful. The prominent ion at m/e 255 ($C_{15}H_{15}N_2O_2$) represented the canthinone portion of 1 after the loss of the tertiary hydroxyl as H_2O. This ion (same composition) was also prominent in the mass spectra of 2 and 3, both of which have the same canthinone portion as in 1. The ion at m/e 411 ($C_{23}H_{27}N_2O_5$) represented the aspidosperma portion of 1. Lactonic alkaloids of the aspidoalbine skeletal type are characterized by prominent ions at m/e 174 ($C_{12}H_{16}N$), 161, 160 ($C_{11}H_{14}N$), 136, and 132, which have been shown[6] to arise from the aliphatic portion of the skeleton after the loss of CO_2 from the lactone function. The mass spectra of 1 (as well as 2 and 3) had these fragments at m/e 172 ($C_{12}H_{14}N$), 159, 158 ($C_{11}H_{12}N$), 134, and 130. The downward shift by 2 mass units indicated the presence of a double bond in the aliphatic part (ring C, D, or E) of the aspidosperma portion of cimilophytine.

The NMR spectrum of 1 was also very revealing and showed some important similarities to those of cimiciphytine and norcimiciphytine. The pattern and location of the four aromatic protons were essentially similar to those of 2 and 3, representing the three adjacent aromatic protons on the canthinone portion of 1 and the lone aromatic proton on the aspidosperma portion. The presence of the strongly hydrogen-bonded hydroxyl at δ 11.49 is analogous to that (δ 11.41) shown by cimiciphytine and represents the proton of the phenol hydrogen bonded to the δ-lactam carbonyl[4] in the canthinone portion of 1. The

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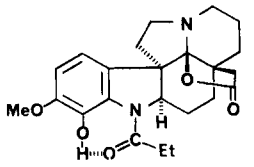
second hydrogen-bonded phenolic proton at δ 10.16 represents a 17-hydroxyl bonded to the carbonyl of the *N*-propionyl function as in cimicidine[3] (**5**). The single



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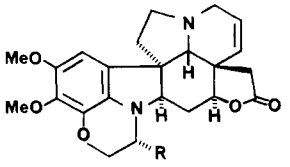
methoxy of **1** must be placed at C_{16} because it is shielded in analogous manner to the shielded C_{16} methoxyls seen in the spectra of its companion alkaloids, cimiciphytine and haplophytine.[1,4]

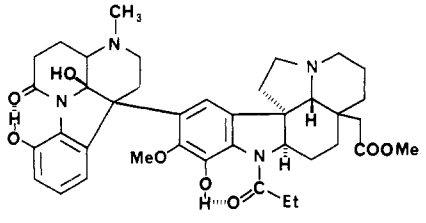
The mass fragmentation pattern of **1** had indicated[6] that the ethylenic double bond in the molecule had to be in ring C, D, or E of the aspidosperma half of **1**. The two vinyl protons in **1** appear in the NMR spectrum as a single peak at δ 5.66, analogous to the vinyl protons of the 6,7-double bonds which were reported[7] to occur as single sharp peak (δ 5.7) in the NMR spectrum of obscurinervine (**6**) or ob-



6 R = CH_2CH_3
7 R = CH_3

scurinervidine (**7**), two lactonic alkaloids of the aspidosperma class. The 2,3-vinyl protons of halophytine (**4**), cimiciphytine (**2**), and norcimiciphytine (**3**), however, occur as an AB quartet with allylic coupling.[1,4]

Cimilophytine, like halophytine,[1] gave on catalytic hydrogenation a zwitterionic tetrahydro derivative formed by the hydrogenolysis of the carbinolamine lactone function and the saturation of the double bond. This indicated that the lactone group of **1** is as in haplophytine and not as in obscurinervine. Catalytic hydrogenation of obscurinervine gave a dihydro derivative by saturation of the 6,7-double bond.[7] Methylation of the tetrahydro derivative obtained by catalytic hydrogenation of **1** gave the methyl ester **8**. The mass spectrum of **8** showed a molecular ion



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at *m/e* 700. The NMR spectrum of **8** showed the new methoxyl of the ester function at δ 3.57 (s, 3 H), but the signal at δ 5.66 (s, 2 H) in the NMR spectrum of **1** was conspicuously absent. A sharp singlet at δ 2.30 (2 H) in the NMR spectrum of **1**, probably representing the methylene group of the lactone function, was also absent in the NMR spectrum of **8**.

The assignment of structure **1** for cimilophytine was clearly supported by an analysis of its ^{13}C NMR spectrum (Table I). Comparison of the ^{13}C NMR spectra[1,3] of cimicidine (**5**), haplophytine (**4**), and cimilophytine (Table I)

Table I. ^{13}C NMR Chemical Shifts of Cimilophytine (**1**), Cimiciphytine (**2**), Haplophytine (**4**), and Cimicidine (**5**)

C	1	2	4	5
2	67.3	72.3	72.0	67.7
3,4	18.5, 21.1	125.7, 130.1	125.5, 130.6	20.0, 25.6
5	41.6	41.6[a]	41.5	40.3
6	132.9	34.5	34.7[c]	33.7[d]
7	125.2	21.3	21.6	25.6
8, 10, 20	43.3, 46.4, 47.5	43.3, 47.3, 47.8	43.5, 47.4, 48.9	42.3, 48.5, 43.5
11	34.3	37.1	35.3[c]	34.8[d]
12	56.7	57.6	57.3	58.0
13	130.5	127.2	126.4	137.2
14	112.1	117.2	118.8	114.5
15	128.9	124.6	124.1	110.3
16	148.2	152.2	151.7	149.9
17, 18	143.6, 147.3	143.7, 147.3	143.9, 149.1	129.6, 128.0
19	104.8	106.9	107.2	106.3
21	174.1	174.7	176.5	175.6
22	171.4			172.2
23	27.5			28.2
24	9.4			9.5
OCH_3	60.7	60.6, 60.7[b]	58.5, 60.9	56.2
NCH_3		35.2, 41.6	35.3	
2′	173.2	171.4	175.1	
3′	34.8	39.0	38.5	
4′	21.1	18.6	22.5	
5′	60.6	60.7[b]	87.5	
7′	51.9	52.1	47.9	
8′	28.6	27.6	29.9	
9′	53.5	53.1	55.1	
10′	124.7	121.3	124.8	
11′	127.2	127.1	128.0	
12′, 13′	117.4, 115.7	117.2, 121.8	117.9, 118.3	
14′, 15′	140.5, 131.9	144.2, 137.7	138.6, 139.0	
16′	95.4	95.6	197.2	
N(6′)CH_3	38.2	41.6[a]	36.6	

[a,b] Superimposed signals. [c,d] These assignments may be reversed.

corroborated the assignment of structure **1** to cimilophytine. Also included in the table is the ^{13}C NMR data of cimiciphytine (**2**), the structure of which was firmly established by chemical correlation with haplophytine.[4] It is noteworthy that the position of the hydroxyl at C-16′ in cimilophytine is supported by correlating its ^{13}C position with that of cimiciphytine (δ 95.4 and 95.6, respectively). The off-resonance-coupled spectrum was often inadequate in determining the multiplicity in some regions of the carbon spectra where the peaks are closely spaced. To overcome this problem, we carried out a spin–echo experiment where a carbon with an odd number of protons gives a negative signal, and with zero or two, a positive signal.[8]

Cimilophytine thus represents a new type of bisindole alkaloid derived by the coupling of an unrearranged canthiphytine unit with a dehydrocimicidine unit.

Experimental Section

General Methods. IR and UV spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer and Perkin-Elmer Model 202 UV–vis spectrophotometer, respectively. Low-resolution mass spectra were recorded on a Hitachi-Perkin-Elmer RMH2 mass spectrometer, and high-resolution mass spectrum

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was recorded on a V.G. Micromass 7070H mass spectrometer. Optical rotation was recorded on a Perkin-Elmer Model 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on either a Bruker WM-250 (at 62.9 MHz for carbon) or an IBM 200SY (at 50.3 MHz for carbon) Fourier transform spectrometer. Chemical shifts (δ) in Table I were measured with respect to $CDCl_3$ (77.00 ppm) and are given with respect to Me_4Si.

Isolation of Cimilophytine. The alkaloidal residue (165 g) after the isolation of haplophytine was subjected to preliminary countercurrent separation in a 30-tube Craig countercurrent machine, between $CHCl_3$ (stationary phase) and McElvain's buffer (moving phase, pH 2.5). The crude base mixture (23.2 g) liberated from the buffer fractions by basification to pH 8.5 with ammonia was resubjected to partitioning in a 200-tube Craig countercurrent machine between $CHCl_3$ and McElvain's buffer, the pH of which was gradually varied from 6.5 to 1.2 over a period of 2 weeks. A total of 1200 × 10 mL fractions were collected. Fractions were combined on the basis of the TLC behavior of the alkaloids from the individual fractions. Fractions 771–800 were combined to give 1.03 g of material, which upon crystallization from $CHCl_3$–EtOH yielded cimilophytine (200 mg): mp 325 °C dec; $[\alpha]^{20}$ −84.9° (EtOH); λ_{max}^{EtOH} 228 (32 800), 266 (15 900), 300 (4000); λ_{max}^{KBr} 3425, 1751, 1621 cm^{-1}; for a proton NMR see text; high-resolution mass measurement, observed m/e 682.3018, $C_{38}H_{42}N_4O_8$ requires m/e 682.3002.

Hydrogenation of Cimilophytine: Formation of Tetrahydrocimilophytine and Its Methyl Ester (8). Cimilophytine (20 mg) in ethanol (8 mL) was hydrogenated over platinum oxide (2 mg) at room terperature and atmospheric pressure. The reaction, monitored by TLC, was stopped after 45 min, CH_2Cl_2 was added, and the mixture was filtered. The filterate gave on concentration gave a polar solid (18 mg); m/e 686 (M^+). Methylation of the solid in methanol with ethereal diazomethane and crystallization of the resulting product from $CHCl_3$–EtOH gave the methyl ester 8 as a colorless solid: mp 270 °C; ^1H NMR δ $CDCl_3$ 1.29 (t, 3 H, J = 7 Hz), 2.35 (s, 3 H), 3.3 (s, 3 H), 3.57 (s, 3 H), 6.29 (d of d, 1 H, J = 7, 1 Hz), 6.85 (d of d, 1 H, J = 8, 1 Hz), 7.04 (d of t, 1 H, J = 8, 1 Hz), 7.33 (s, 1 H), 7.54 (s, 1 H), 10.69 (s, 1 H), 11.38 (s, 1 H); MS, m/e (relative intensity) 700 (73, M^+), 682 (6, $M^+ - 18$), 669 (22, $M^+ - 31$), 657 (52), 429 (3), 255 (16), 168 (9).

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Microbially Mediated Enantioselective Ester Hydrolyses Utilizing *Rhizopus nigricans*. A New Method of Assigning the Absolute Stereochemistry of Acyclic 1-Arylalkanols

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The fungus *Rhizopus nigricans* has been used to effect the enantioselective hydrolysis of 22 acetates of acyclic 1-arylalkanols. The absolute stereochemistry of all the chiral alcohols formed can be accounted for by a rule which is based on the relative sizes of substituents on the carbinol carbon. The relative sizes of substituents deduced from these hydrolyses have been compared with those assigned from Horeau's method for the same compounds and found to be identical; i.e., a phenyl group or heterocyclic ring is always larger than an alkyl group. The use of *R. nigricans* to prepare chiral alcohols and use of the rule to predict their configuration constitute a new method of assigning the absolute stereochemistry of secondary alcohols. With minor modifications this method can also be used to prepare gram quantities of chiral alcohols of both configurations.

Chiral secondary alcohols of established absolute stereochemistry are remarkably versatile intermediates for the synthesis of a host of compounds, since there are many stereospecific reactions available for replacing the hydroxyl group with halogen, nitrogen, sulfur, or carbon. In order to take advantage of this versatility, we worked on the asymmetric synthesis of alcohols whose absolute stereochemistry would be predicted from the method of preparation. While there are purely chemical methods for preparing alcohols of high optical purity, the absolute stereochemistry of the products is not always predictable. There are, however, a number of enzymic or microbial methods which exhibit the desired product stereoselectivity. For example, Jones et al.[2] have recently described the use of horse liver alcohol dehydrogenase (HLAD) for the synthesis of a variety of alcohols with predictable configurations. Prelog and co-workers[3] carried out pioneering studies on the use of an oxido reductase present in *Curvularia falcata* to prepare chiral alcohols. From these studies they proposed a rule, based on the relative sizes of substituents flanking the carbonyl group to be reduced, which accounts for their observations. Prelog's rule was later shown also to rationalize the configurations of a variety of aromatic and heteroaromatic alcohols formed from the corresponding ketones and *Cryptococcus macerans* or *Sporobolomyces pararoseus*.[4] While this

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