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Cissampentin: A New Bisbenzylisoquinoline Alkaloid from *Cissampelos fasciculata*

Deborah L. Galinis,[a] David F Wiemer,[a*] and John Cazin, Jr.[b]

Departments of Chemistry[a] and Microbiology[b]
University of Iowa
Iowa City, Iowa 52242

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Abstract: A new bisbenzylisoquinoline alkaloid, cissampentin, has been isolated from the aerial parts of *Cissampelos fasciculata* Detailed interpretation of various spectra allowed identification of most structural features, including a rare methyleneoxy bridge Although attempted methylation of this alkaloid led to complex mixtures, reaction with diethyl phosphorochloridate gave a single diethyl phosphate derivative and allowed assignment of a 7'-11 ether linkage Bioassays indicate significant activity as a repellent to the leafcutter ant *Acromyrmex octospinosus*, and limited antifungal activity

Although leafcutter ants harvest material from a broad range of the plant species found in the tropical Americas, some species appear to be defended against leafcutter ant attack by the presence of novel secondary metabolites.[1] During the course of our studies on some such plants, we have isolated a number of biologically active compounds, primarily terpenoids [2] In this paper we describe our investigation of *Cissampelos fasciculata*, work that has led to identification of a new bisbenzylisoquinoline alkaloid [3]

Results and Discussion

Aerial parts of *Cissampelos fasciculata* Benth (Menispermaceae) were collected in Costa Rica after our field bioassay[4] suggested that fresh leaves were highly repellent to a local leafcutter ant, *Acromyrmex octospinosus* (Reich) Because the plant belongs to a family known to produce alkaloids,[5] a Mayer's test was performed on an EtOH extract When this test proved positive, a portion of the remaining extract was subjected to a standard alkaloid extraction sequence Radial dispersion chromatography on the recovered material gave two main fractions One contained only pure compound 1, a new compound we have named cissampentin, while the second fraction gave the known alkaloid corydine (3) after further purification by reverse phase HPLC

The molecular formula of cissampentin was established as $C_{37}H_{40}N_2O_6$ by high resolution mass spectrometry (*m/z* 608.2932 $[M]^+$), and requires 19 degrees of unsaturation The ^{13}C NMR spectrum implied that sixteen degrees of unsaturation could be attributed to four aromatic rings, accounting for all of the ^{13}C resonances greater than 100 ppm The absence of any other sp^2 carbons suggested that the remaining three degrees of unsaturation were due to three additional ring systems Thirteen non-aromatic carbons could be characterized by ^{13}C NMR and DEPT experiments (Table 1) Four of these signals represent methyl carbons (two N-methyl and two O-methyl groups) while the other resonances implied seven methylene units (four simply aliphatic, two bearing nitrogen, and one bearing oxygen) and two

methine units (both bearing nitrogen) These observations led to the hypothesis that cissampentin contained a bisbenzylisoquinoline skeleton

An interesting and unusual feature of the ^{1}H NMR spectrum is the pair of geminally coupled resonances at δ 4.57 and 5 06, suggesting a methyleneoxy bridged alkaloid.[6] Comparison of the proton and carbon chemical shifts of the methyleneoxy bridge with those reported for cycleatjeheninе[6b,c] confirmed a bisbenzylisoquinoline alkaloid with this relatively rare structural feature Its location in the molecule was fixed by a combination of NMR experiments One of the four aromatic rings was identified as para substituted based on a pattern arising from resonances at δ 7 08 and δ 7 31 in the ^{1}H NMR spectrum (Table I) An HMBC[7] experiment revealed long range coupling between one of these aromatic resonances (δ 7.08) and a carbon resonance at 77.66 ppm. These data, and a correlation between the aromatic resonance at δ 7.31 and a carbon resonance (36 28 ppm) representing the second substituent on this ring, led to assembly of partial structure **A**

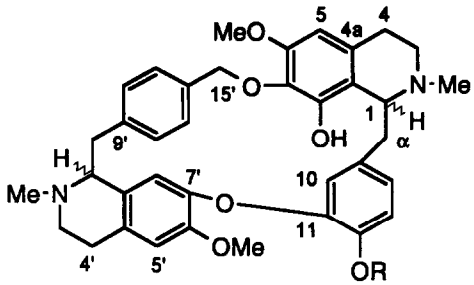
Partial structures **B** and **C** also were indicated by coupling patterns in the ^{1}H NMR spectrum and ^{1}H-^{13}C long range coupling in the HMBC spectrum The distinctive coupling pattern of the ^{1}H resonances at δ 6 78, 6 85, and 6 91 and a correlation of these three protons with two oxygenated aromatic carbons were attributed to partial structure **B** In contrast, the two aromatic H's assigned to partial structure **C** appeared as singlets in the ^{1}H NMR spectrum. This observation, together with several ^{1}H-^{13}C correlations (Table 1), led to the para relationship expressed in **C** The final aromatic ring must be pentasubstituted, bearing three oxygen substituents, two carbons, and a sole hydrogen corresponding to the ^{1}H resonance at δ 6.21 The observed correlation of this resonance and signals at 132 39 and 150 46 ppm, along with a correlation between signals at δ 5 06 and 4.58 and the 132.39 resonance, allowed assembly of partial structure **D**, representing the fourth aromatic ring and its attachment to the **A** fragment

In the next phase of the structure elucidation, two heterocyclic rings were identified, one attached to the **D** fragment and the second to structure **C** A ^{1}H-^{1}H COSY experiment allowed identification of a -CH_2CH_2- group and a methine further coupled to a benzylic methylene group Information from the HMBC and HMQC spectra indicated that these units connect an N-Me group to the aromatic ring, forming a six-membered ring as shown in structure **E** and leaving the remaining aromatic positions for oxygen substituents In a similar fashion, a six-membered ring including an N-Me group could be added to partial structure **C**, leading to the larger subunit **F** For example, correlation of one aromatic resonance in **C** (δ 6.63) with the -CH_2- resonance at 29 40 ppm supports this assignment

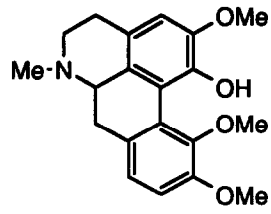
Partial structures **B** and **F** were connected to partial structure **E** based primarily on HMBC data Correlations of an aromatic resonance (δ 6 78) with the methylene at 40 50 ppm, previously connected to the heterocyclic ring of **E**, defined the connectivity of structures **B** and **E** Furthermore, the hydrogens of the benzylic carbon originally assigned in **A** (36 28 ppm) were coupled with the methine H of the **F** subunit, allowing attachment of the second heterocyclic ring to partial structure **E**

A variety of experiments was used to assign the oxygenated substituents and the second ether linkage of this complex alkaloid Strong NOE's observed between the resonances at δ 6 21 and 3 87, and between signals at δ 6 63 and 3 79, allowed assignments of the two methoxy groups With both methoxy positions assigned, the third oxygen substituent on the aromatic ring of fragment **B** must be an -OH group

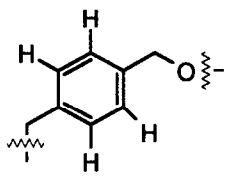
The issue of connectivity, i e whether the macrocyclic ring involved a 7'-11 or a 7'-12 ether linkage, was difficult to establish by direct spectroscopic means Attempts to methylate cissampentin with



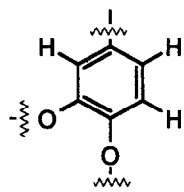
1 R = H
2 R = $PO(OEt)_2$



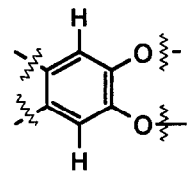
3



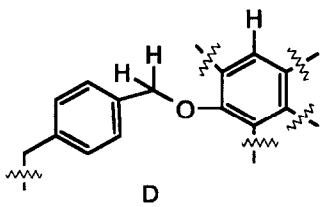
A



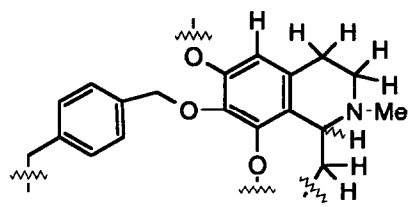
B



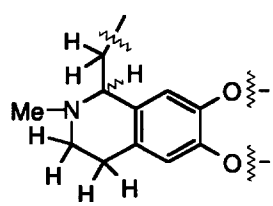
C



D



E



F

Table I ^{1}H and ^{13}C NMR Data for Cissampetin

C/H	^{13}C	^{1}H	long range ^{1}H-^{13}C correlations
1	61.26 (d)	3 58 (br d, 8.2)	α, 3, 8
3	44.99 (t)	2 81 (m)	1, 4, 8a
		3.25 (td, 12.1, 4.7)	
4	24.16 (t)	2.86 (m)	5, 4a, 8a
		2.88 (m)	
4a	119.23 (s)		
5	102.80 (d)	6 21 (s)	4, 6, 7
6	150.46 (s)		
7	132 39 (s)		
8	146 54 (s)		
8a	129 66 (s)[a]		
9	134 69 (s)		
10	117 97 (d)	6.78 (s)	α, 11, 14
11	144 23 (s)		
12	144 85 (s)		
13	115 01 (d)	6.85 (d, 7 9)	9, 11
14	124.40 (d)	6 91 (d, 7 9)	10, 12
α	40 50 (t)	2.71 (m)	1, 9, 10, 14
N2-Me	42.86 (q)	2.27 (s)	
6-OMe	55.72 (q)	3 87 (s)	6
8-OH		4 84 (s)	
1'	63.62 (d)	3.65 (br s)	α', 8a'
3'	52 47 (t)	2 55 (m)	1'
		3 09 (m)	
4'	29 40 (t)	2.63 (dd; 14.8, 2 6)	5'
		3 09 (m)	
4a'	129.50 (s)[a]		
5'	112.37 (d)	6.63 (s)	4', 6', 7'
6'	148 50 (s)		
7'	143 33 (s)		
8'	117 01 (d)	6 79 (s)	1', 6', 7'
8a'	139.49 (s)		
9'	134 82 (s)		
10'	130 19 (d)	7 31 (d, 7 8)	α', 9', 11', 14'
11'	128 64 (d)	7.08 (d; 7.8)	10', 12', 15'
12'	139 49 (s)		
13'	128.64 (d)	7 31 (d, 7.8)	
14'	130 19 (d)	7 08 (d, 7 8)	
15'	77 66 (t)	5 06 (d, 12 1)	7, 11' 12', 13'
		4 58 (d, 12 1)	
α'	36 28 (t)	3.04 (dd, 16 8, 4 1)	4a', 8a'
		3.36 (dd, 16 8, 4 1)	
N2'-Me	43.65 (q)	2 48 (s)	
6'-OMe	55 64 (q)	3 79 (s)	6'

[a] Assignments may be interchanged

diazomethane gave only decomposition, consistent with a previous report[6] indicating low yield methylation of a related alkaloid. Attempted reaction with methyl iodide also gave a complex mixture. However, phosphorylation of the phenol proved possible upon reaction with diethyl phosphorochloridate, and gave a single monophosphate that was readily purified. In this phosphate derivative (2), a dramatic change (ca 0 5 ppm) in the chemical shift of H-13 allowed assignment of cissampentin as the 7'-11 isomer as shown in structure 1 Unfortunately, because cissampentin itself has been observed only as an oil, and we have not yet been able to obtain a crystalline derivative, it is not possible to assign the C-1 and C-1' stereochemistry at this time. Finally, no rotation was observed suggesting that cissampentin is racemic.[6c]

Compound 3, an unrelated alkaloid, also was isolated from this plant extract. The EI mass spectrum showed a molecular ion at m/z 341, corresponding to a composition of $C_{20}H_{23}O_4N$. The ^1H and MS spectra of compound 3 were identical to those reported for the aporphine alkaloid corydine.[9] Both the ^{13}C and DEPT spectra supported this assignment

The bioactivity of cissampentin has been studied using a captive colony of *Acromyrmex octospinosus*. When tested at the minimal natural concentration (0 57 mg/g of wheat flakes) the ants demonstrate a significant preference for control over test wheat flakes (30/11; $p < 0.005$) [4] Cissampentin also shows some limited inhibition of the ant garden fungus at concentrations of 5 and 10 μg/mL,[10, 11] supporting the hypothesis that some plants are defended by leafcutter attack by the presence of anti-fungal compounds

While cissampentin belongs to the common bisbenzylisoquinoline family, it is interesting both for the presence of the methyleneoxy bridge[6] form and for its biological activity. These features should encourage studies of related plants from Central America

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Experimental

The NMR spectra (^1H and ^{13}C) were recorded on a Bruker AMX-600 instrument in $CDCl_3$ solutions with an internal TMS standard. Both low and high EIMS were obtained at 70 eV

Plant Collection. *Cissampelos fasciculata* Benth was collected at Rincòn de la Vieja, air dried at ambient temperature, and stored in plastic bags until extracted

Isolation Air-dried aerial parts of *Cissampelos fasciculata* Benth (225 g) were ground in a Waring blender, and then steeped with EtOH (2 5 L) After concentration of the EtOH extract *in vacuo*, ca 12 3 g of residue remained Approximately 7.84 g of the residue was further extracted with 10% HCl, and then filtered The filtrate was extracted with ether, and the ether layer was discarded The aqueous layer was made basic with NH_4OH, and then was extracted with CH_2Cl_2 After the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, about 225 mg of crude alkaloid mixture was obtained. Radial dispersion chromatography with a MeOH/$CHCl_3$ gradient (5-40% MeOH), gave compound 1 in pure form (80 mg) corresponding to 0.056% of the dried plant A second fraction gave compound 3, which was

further purified by reverse phase HPLC with MeOH/H_2O (80/20%) on a C-18 column (8 μ DYNAMAX column; 10 mm ID x 25 cm) affording 5 5 mg (0 004%)

Cissampentin (1) yellow oil; $[\alpha]^{25}_D$ = 0 0° (c = 0.002, CH_2Cl_2); ^1H and ^{13}C NMR data cf Table I; EIMS *m/z* (relative intensity) 608 (1), 400 (3), 298 (100), 266 (15), 206 (29), 161 (20), 132 (7), 104 (3), 77 (2); HREIMS calcd $C_{37}H_{40}N_2O_6$ 608 2876, found 608.2932

Phosphate 2. To cissampentin (10.8 mg, 0 02 mmol) in benzene (0.5 mL) was added diethyl phosphorochloridate (2.88 μl, 0.04 mmol) followed by one drop of Et_3N After standing overnight at 40 °C, the resulting solution was purified by radial dispersion chromatography (silica gel, MeOH/$CHCl_3$ gradient) to give 1.3 mg (8 5%) of phosphate **2** ^1H NMR, δ 7 31 (2H, d, J = 7 8 Hz, H-10, H-14), 7.23 (2H, d, J = 7 8 Hz, H-11', H-13'), 7.03 (2H, d, J = 7 9 Hz, H-13, H-14), 6.84 (1H, s, H-10), 6 79 (1H, s, H-8'), 6 59 (1H, s, H-5'), 6.22 (1H, s, H-5), 5 06 (1H, d, J = 12 1 Hz, H-15'), 4 73 (1H, s, 8-OH), 4 52 (1H, d, J = 12 1 Hz, H-15'), 4 26 (4H, m, -$(OEt)_2$), 3 87 (3H, s, 6-OMe), 3.49 (3H, s, 6'-OMe), 3 65 (1H, br s, H-1'), 3.58 (1H, m, H-1), 3 41 (1H, dd, J = 16 4, 3 1 Hz, H-α'), 3.26 (1H, m, H-3), 3 05 (2H, m, H-3', H-4'), 2.95 (1H, dd, J = 16 8, 3 1 Hz, H-α'), 2 86 (2H, m, H-4), 2 82 (1H, m, H-3), 2 71 (2H, m, H-α), 2 56 (1H, m, H-4'), 2.52 (1H, m, H-3'), 2 50 (3H, s, N2'-Me), 2 27 (3H, s, N2-Me), 1 34 (3H, t, J = 7.03, -OEt), 1 31 (3H, t, J = 7.03, -OEt),^{31}P NMR, δ -6.68.

Corydine (3). Brown gum; $[\alpha]^{25}_D$ = +65.42° (c = 0 005, EtOH), lit[9] $[\alpha]^{25}_D$ = +204° (c = 0 6, EtOH); ^1H NMR identical to literature data;[9] ^{13}C NMR 152 27 (s), 149.65 (s), 144.29 (s), 142 80 (s), 131.11 (s), 128 18 (s), 128 09 (s), 126 86 (s), 124.77 (d), 124.21 (s), 119 66 (s), 111 77 (d), 111 33 (d), 63 02 (d), 62 39 (q), 56 44 (q), 52 96 (t), 43 99 (q), 35 69 (t), 29.07 (t), EIMS m/z(relative intensity) 341 (56), 323 (29), 310 (100), 279 (10), 155 (28), 133 (14), 125 (8)

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