Pergamon

Tetrahedron Letters 41 (2000) 2843–2846

TETRAHEDRON LETTERS

Cavanine, a novel α-hydroxybisbenzylisoquinoline alkaloid from *Sciadotenia toxifera* (Krukoff and A. C. Smith)

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Received 20 January 2000; revised 10 February 2000; accepted 11 February 2000

Abstract

Sciadotenia toxifera (Krukoff and A. C. Smith), a member of the South American Menispermaceae, has been used in folk medicine for the treatment of malaria and as an ingredient of curare. From the basic fraction of this bush-rope, a new bisbenzylisoquinoline alkaloid, cavanine, was isolated. The novel structure of cavanine (**1**), incorporating a rare α-hydroxyl substituent on one of the benzylisoquinoline moieties, was completely determined by spectroscopic methods.

Keywords: alkaloids; bisbenzylisoquinolines; natural products; Menispermaceae.

South American Menispermaceae have achieved a distinguished place in medicine because some of these species, used by indigenous people, were determined to possess curare activity.[1] *Sciadotenia toxifera* (Krukoff and A. C. Smith), a Peruvian Menispermaceae which grows as a bush-rope, is one such species which has also been used in folk medicine for treatment of sterility and malaria.[2,3] Two minor alkaloids, sciaferine and *N*-formylanalobine, have recently been isolated from the neutral fraction of this species.[4] Four known bisbenzylisoquinolines, (+)-sciadenine, (+)-sciadoline (**2**), (+)-sciadoferine, and (+)-isochondodendrine, were also identified from the basic fraction of this bush-rope.[3,5,6] Even though the total bases were found to be anti-tumor active by NCI testing, the active compound(s) was not able to be isolated.[5,6]

In a continued search for the active component among the total bases, gradient pH extractions were conducted over a pH range of 6.0 to 12.0. The extracts from pH range 6 and 8 were combined and further subjected to column chromatography on silica gel. The fraction eluting with 5% CH_3OH in CH_2Cl_2, upon further purification with silica gel chromatography and crystallization from CH_3OH/CH_2Cl_2, provided colorless needles of the novel bisbenzylisoquinoline alkaloid, cavanine (**1**), an α-hydroxyl analog of sciadoline.

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0040-4039/00/$ - see front matter
PII: S0040-4039(00)00299-9

The IR data for cavanine indicated the presence of hydroxyl (3370 cm^{-1}) and the bathochromic shift in the UV spectrum with NaOH showed the presence of phenolic hydroxyl group.[7] The DCI-CH_4 mass spectrum of **1** revealed a molecular weight of 606 daltons, and the DCI-ND_3 mass spectrum indicated that there were two exchangeable hydrogens present. The molecular formula of **1** was determined to be $C_{36}H_{34}N_2O_7$ by HRDCIMS (CH_4 reagent gas), requiring 21 degrees of unsaturation.

The ^1H NMR spectrum of **1** dissolved in $CDCl_3$:CD_3OD (9:1) revealed only 32 of the 34 total hydrogens because two hydrogens had exchanged with deuteria from the CD_3OD solvent. The ^{13}C GASPE NMR spectrum, which is summarized along with the hydrogen data in Table 1, revealed the presence of 15 quaternary, 14 methine, three methylene and four methyl signals for a total of 36 carbon resonances. Collectively the data suggested that **1** was a bisbenzylisoquinoline alkaloid containing three methoxyl and one *N*-methyl substituents. The presence of only two methine and three methylene carbon signals in the aliphatic region suggested that the A ring of one of the benzylisoquinoline moieties was fully aromatic while the other moiety was a tetrahydrobenzylisoquinoline unit. The presence of two aliphatic methine carbons, one of which resonated at δ 73.7, further indicated that one of the alpha carbons bore an oxygenated substituent.

Table 1
^1H (400 MHz) and ^{13}C (100 MHz) NMR assignments in $CDCl_3$:CD_3OD (9:1) for **1**

C#	δ_C (mult)	δ_H (mult, J, integral)	C#	δ_C (mult)	δ_H (mult, J, integral)
1	155.7 (s)		1'	58.4 (d)	4.10 dd (1.1, 11.4 Hz, 1H)
3	137.7 (d)	8.30 d (5.6 Hz, 1H)	3'	44.4 (t)	3.17 m (1H)
4	120.3 (d)	7.51 d (5.6 Hz, 1H)			2.77 m (1H)
4a	132.7 (s)		4'	24.0 (t)	2.90 m (2H)
5	102.5 (d)	7.02 (1H)	4a'	129.7 (s)	
6	152.1 (s)		5'	109.8 (d)	6.56 (1H)
7	139.4 (s)		6'	152.1 (s)	
8	136.0 (s)		7'	139.4 (s)	
8a	118.0 (s)		8'	143.6 (s)	
α	73.7 (d)	6.38 (1H)	8a'	124.4 (s)	
9	134.9 (s)		α'	37.5 (t)	3.03 dd (1.1, 13.3 Hz, 1H)
10	125.6 (d)	6.13 dd (2.3, 8.6 Hz, 1H)			2.49 dd (11.4, 13.3 Hz, 1H)
11	115.4 (d)	6.38 dd (2.3, 8.6 Hz, 1H)	9'	130.2 (s)	
12	156.4 (s)		10'	129.3 (d)	6.50 dd (2.3, 8.6 Hz, 1H)
13	113.8 (d)	5.64 dd (2.3, 8.6 Hz, 1H)	11'	117.3 (d)	6.66 dd (2.3, 8.6 Hz, 1H)
14	133.0 (d)	6.87 dd (2.3, 8.6 Hz, 1H)	12'	156.5 (s)	
OMe-6	56.1 (q)	3.94 (3H)	13'	114.2 (d)	5.29 dd (2.3, 8.6 Hz, 1H)
			14'	129.4 (d)	6.52 dd (2.3, 8.6 Hz, 1H)
			NMe-2'	42.2 (q)	2.25 (3H)
			OMe-6'	55.8 (q)	3.76 (3H)
			OMe-7'	60.0 (q)	3.40 (3H)

Of the 21 degrees of unsaturation indicated by the molecular formula, the fully aromatic benzylisoquinoline moiety accounted for 11 while the tetrahydrobenzylisoquinoline moiety accounted for another nine. The remaining degree of unsaturation suggested that the monomers were connected by two bridges forming an internal macrocycle.

COSY NMR data allowed identification of the members of the individual spin systems in **1**, and NOESY NMR data provided evidence for the spatial relationship between these spin systems. By these means the aromatic doublets representing H-3 at δ 8.30 (J=5.6 Hz) and H-4 at δ 7.51 in ring A (see

Fig. 1) were identified. The H-5 aromatic singlet at δ 7.02 displayed NOESY correlations to H-4 and to OCH_3-6 at δ 3.94 in ring B. Supported by heteronuclear correlation data, the assignments for the fully aromatic benzylisoquinoline moiety are summarized in Table 1. Of particular interest was the chemical shift of the α methine carbon resonance at δ 73.7, clearly establishing the presence of an oxygen atom attached in this unique position.

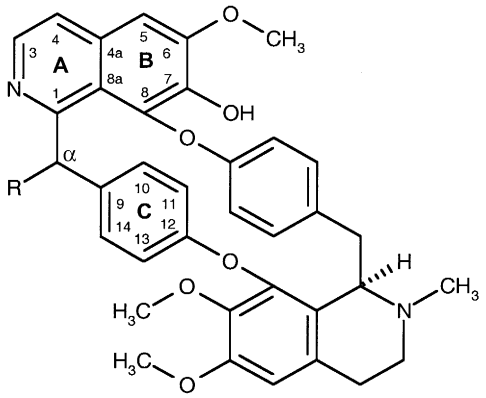

Fig. 1. Structure with R=OH, cavanine (**1**) and R=H, sciadoline (**2**)

The remaining *N*-methylcoclaurine tetrahydrobenzylisoquinoline monomer (the right-hand portion of the structure represented in Fig. 1 labeled with primed numbers) contained aliphatic H-3′ multiplets at δ 3.17 and δ 2.77 and H-4′ multiplets at δ 2.90 in ring A′. The H-5′ aromatic singlet at δ 6.56 displayed NOESY correlations to H-4′ and to OCH_3-6′ at δ 3.76 in ring B′. HMBC correlations established that the sole remaining methoxyl group at δ 3.40 was attached to C-7′. The *N*–CH_3-2′ singlet at δ 2.25 likewise correlated with the adjacent H-3′ resonances as well as with the H-1′ methine doublet of doublets at δ 4.10 in the NOESY spectrum. Supported by heteronuclear correlation data, the assignments for the tetrahydrobenzylisoquinoline moiety are summarized in Table 1.

At this point the sites of attachment of the two hydroxyl groups and the connection sites of the two ether bridges binding the monomers together remain to be determined. DCI-ND_3 mass spectral fragmentation data established that the two exchangeable hydroxyl groups were both situated on the fully aromatic benzylisoquinoline unit, and furthermore, neither hydroxyl group was situated on ring C. A small portion of compound **1** was treated with diazomethane, and the resulting mass spectral parent ion increased by 14 daltons (CH_2), clearly indicating the presence of one phenolic group.

This mono-methylated product was further treated with TFA anhydride in an attempt to acetylate any remaining alcohols. The resulting mass spectral parent ion increased by an additional 96 daltons (CF_3CO), confirming the presence of one non-aromatic alcohol, i.e. the alcohol group attached to the α carbon atom. Therefore, it was concluded that the two ether bridges connected C-12 and either C-7 or C-8 in the benzylisoquinoline moiety to C-12′ and C-8′ in the tetrahydrobenzylisoquinoline moiety.

A key NOESY correlation between H-13 in the tail of ring C and the methoxyl group attached at C-7′ in ring B′ established that one of the ether bridges in **1** was situated between C-12 and C-8′ in a head-to-tail fashion. Other NOESY correlations were consistent with the remaining bridge existing between C-8 and C-12′, and the resulting structure for cavanine (**1**) is shown in Fig. 1. The stereochemistry of the α-alcohol was not determined. Bargustanine,[8] isolated from the roots of *Berberis vulgaris*, incorporates an α-hydroxytetrahydrobenzylisoquinoline moiety attached to a tetrahydroisoquinoline unit via a head-to-head ether linkage. To our knowledge, cavanine is the first natural α-hydroxybisbenzlisoquinoline alkaloid to be reported in the literature.

Acknowledgements

We are grateful to Dr. Michael P. Cava, University of Alabama, for supplying the plant material.

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7. Cavanine (**1**) colorless needles (17.6 mg, CH_3OH/CH_2Cl_2): IR (KBr) 3370, 1610, 1563, 1500 cm^{-1}; UV (95% EtOH) λ_{max} (log ε) 242 (4.10), 259sh (4.03), 276 (3.77), 290sh (3.09), 328sh (3.50), 336 (3.58) nm; UV (95% EtOH+0.1 M NaOH) λ_{max} (log ε) 238 (4.18), 244 (4.19), 248 (4.18), 287 (4.18), 370 (4.16) nm; ^{1}H NMR ($CDCl_3$:CD_3OD 9:1, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$:CD_3OD 9:1, 100 MHz) see Table 1; LRDCIMS(CH_4) *m/z:* $[M+H]^+$ 607 (97), 589 (100), 575 (5), 501 (2), 107 (7); HRDCIMS (CH_4) *m/z* 606.2353 (calcd for $C_{36}H_{34}N_2O_7$ 606.2366).
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