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Ermias Dagne, A. A. Leslie Gunatilaka, David G. I. Kingston, and Melaku Alemu

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4′-*O*-METHYLSTEPHAVANINE FROM *STEPHANIA ABYSSINICA*

ERMIAS DAGNE,[1] A.A. LESLIE GUNATILAKA, DAVID G.I. KINGSTON,*

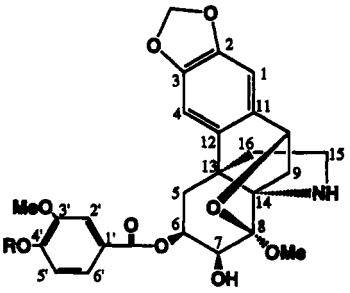
Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

and MELAKU ALEMU

Department of Chemistry, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia

ABSTRACT.—An EtOH extract of the roots of *Stephania abyssinica* (Menispermaceae) contained stephavanine [**2**] and a new alkaloid, 4′-*O*-methylstephavanine [**1**].

Stephania abyssinica Walp. (Menispermaceae) is a creeper indigenous to southern and eastern Africa. The leaves of this plant are used as a purgative and emetic, whereas the roots are employed in the treatment of roundworm, menorrhagia, and boils (1). Previous phytochemical investigations of the plant material collected from Ethiopia and South Africa have revealed the presence of seven hasubanan alkaloids including stephavanine [**2**] (2,3). During our random screening of plant extracts for anticancer activity using a mechanism-based bioassay with DNA repair-deficient and repair-proficient yeast mutants (4), MeCOEt and EtOH extracts of *S. abyssinica* roots exhibited moderate activities. Several attempts to isolate the bioactive constituent(s) resulted in significant loss of bioactivity. However, our fractionation studies led to the isolation of two ester-ketal hasubanan alkaloids, 4′-*O*-methylstephavanine [**1**] and stephavanine [**2**], of which the former is new. Structure elucidation of **1** and assignments of ^{1}H- and ^{13}C-nmr spectral data of both **1** and **2** are presented in this paper.



1 R=Me
2 R=H

The EtOH extract of *S. abyssinica* roots was partitioned with $CHCl_3/H_2O$, and the bioactive $CHCl_3$ fraction was subjected to cc over Si gel. Elution with $CHCl_3$/MeOH mixtures of increasing polarities afforded several fractions, none of which possessed significant bioactivity. However, on tlc examination, the column fraction eluted with 5% MeOH in $CHCl_3$ revealed the presence of a major constituent. Further purification of this fraction by Sephadex gel permeation chromatography followed by preparative tlc gave the alkaloid **1**.

Compound **1** had the composition $C_{27}H_{29}NO_9$ as deduced from its nmr and hrms data. The ^{13}C-nmr spectrum displayed 27 carbon resonances, corresponding to three primary, five secondary, eight tertiary, and eleven quarternary carbon atoms. These data together with its ^{1}H-nmr spectrum (Table 1) suggested that **1** was a member of the hasubanan family of alkaloids carrying an aroyl ester substituent at C-6 (5). The ^{1}H-nmr spectrum also indicated a CH_2O_2 and three MeO functions; the pattern due to aromatic protons indicated tetrasubstituted and trisubstituted aromatic rings. The presence of the

[1]Present Address: Department of Chemistry, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia.

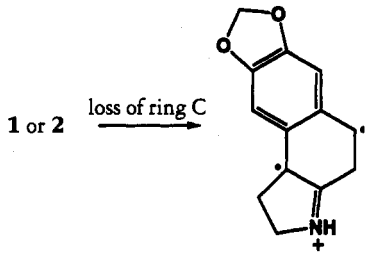
TABLE 1. ^{1}H- and ^{13}C-nmr Data for **1** and **2** in $CDCl_3$.[a]

Position	Compound			
	1		**2**	
	^{1}H-nmr[b]	^{13}C-nmr[c]	^{1}H-nmr[b]	^{13}C-nmr[c]
1	6.45 s	107.3 (d)	6.46 s	107.2 (d)
2	—	147.8 (s)	—	147.6 (s)
3	—	144.8 (s)	—	144.7 (s)
4	6.47 s	106.7 (d)	6.48 s	106.5 (d)
5α	2.32 dd (3.0, 15.1)	35.4 (t)	2.32 dd (3.0, 15.0)	35.4 (t)
5β	2.56 dd (3.4, 15.1)		2.53 dd (3.0, 15.0)	
6	5.14 m	72.0 (d)	5.15 m	72.0 (d)
7	4.23 d (4.2)	73.2 (d)	4.28 d (4.2)	73.1 (d)
8	—	102.0 (s)	—	101.9 (s)
9α	1.93 d (10.8)	38.9 (t)	1.93 d (11.0)	38.9 (t)
9β	2.52 dd (5.1, 10.8)		2.46 dd (5.1, 11.0)	
10	4.83 d (6.3)	77.3 (d)	4.82 d (5.1)	77.2 (d)
11	—	136.2 (s)	—	136.3 (s)
12	—	132.9 (S)	—	133.0 (s)
13	—	47.2 (s)	—	47.0 (s)
14	—	77.3 (s)	—	77.2 (s)
15	2.02 m	39.1 (t)	2.00 m	39.1 (t)
16	3.18 m	41.3 (t)	3.15 m	41.3 (t)
1′	—	122.0 (s)	—	121.6 (s)
2′	7.27 d (1.9)	109.7 (d)	7.25 d (2.0)	113.3 (d)
3′	—	148.2 (s)	—	149.8 (s)
4′	—	152.9 (s)	—	145.6 (s)
5′	6.68 d (8.4)	112.1 (d)	6.75 d (8.5)	111.6 (d)
6′	6.97 dd (1.9, 8.4)	123.7 (d)	6.92 dd (2.0, 8.5)	124.2 (d)
8-OMe	3.61 s	52.2 (q)	3.60 s	52.0 (q)
3′-OMe	3.92 s	56.2 (q)	3.92 s	56.2 (q)
4′-OMe	3.91 s	56.2 (q)	—	—
-OCH_2O-	5.06 d, 5.67 d (1.4)	100.8 (t)	5.09 d, 5.69 d (1.5)	100.7 (t)
ester C=O	—	165.7 (s)	—	165.6 (s)

[a]Chemical shifts are in ppm from internal TMS.
[b]At 400 MHz; *J* values in Hz are given in parentheses.
[c]At 100.57 MHz; multiplicities determined by a DEPT sequence.

base peak at *m/z* 214 in the ms of **1** due to the fragment **A** (Scheme 1) indicated the attachment of the CH_2O_2 substituent to ring A (6). In the ^{1}H-nmr spectrum of **1** the signals due to the CH_2O_2 protons appeared as two doublets (J=1.4 Hz) at δ 5.67 and 5.06, pointing to the close proximity of the aromatic ring of the veratryl ester to the CH_2O_2 protons. The value of the coupling constants between each of the C-5 methylene protons and the C-6 proton (J=3.02 and 3.45 Hz) were indicative of either axial-equatorial or equatorial-equatorial coupling, thus suggesting that the veratryl moiety at C-6 has β-axial and the OH on C-7 has β-equatorial orientation (5,7). On the basis of the foregoing evidence, **1** was identified as 8,10-epoxy-7-hydroxy-8-methoxy-2,3-[methylenebis(oxy)] hasubanan-6-(3,4-dimethoxybenzoate) or 4′-*O*-methylstephavanine, the *O*-methyl derivative of a previously reported hasubanan alkaloid, stephavanine [**2**]. [The chemical literature (8,9) contains a reference to "methylstephavanine," but this is really *N,O,O*-trimethylstephavanine and thus differs from compound **1**.]

Fractionation of the MeCOEt extract by gel permeation chromatography followed by preparative tlc also caused a loss of bioactivity but yielded a single pure



A
m/z 214

SCHEME 1

compound. The physical and spectral data of this compound indicated it to be stephavanine [**2**], an alkaloid previously encountered in the same plant species (10). The structure and stereochemistry of **2** have been determined by X-ray crystallography. Although ^{1}H-nmr spectral data of several derivatives of stephavanine [**2**] have been reported (10), to the best of our knowledge neither the ^{1}H-nmr nor the ^{13}C-nmr spectral data of the parent alkaloid have been published. In this paper we have assigned the ^{1}H- and ^{13}C-nmr spectral data of both **1** and **2** (Table 1); these assignments are based on comparison with published assignments for known hasubanan alkaloids (9).

Both 4′-*O*-methylstephavanine [**1**] and stephavanine [**2**] were found to be inactive in our mechanism-based anticancer bioassay (4). We are currently engaged in the isolation of the biologically active compound(s) present in *S. abyssinica*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Unless otherwise stated, instrumentation, general isolation, and bioassay procedures were the same as those described in our previous publication (4). Uv spectra were recorded for MeOH solutions on a Beckman DU-50 spectrophotometer.

PLANT MATERIAL.—Roots of *S. abyssinica* (S-147) were collected from central Ethiopia 2 km from the two of Menagesha, altitude 2350 m, in April 1988. A voucher specimen under the cipher ED-S425 was deposited in the National Herbarium, Addis Ababa University, Ethiopia.

EXTRACTION AND ISOLATION.—Dried and powdered roots of *S. abyssinica* (450 g) were extracted with EtOH at room temperature by percolation for 2 days. Removal of EtOH yielded a dark oily residue (17 g), which was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ fraction (10 g) was subjected to cc over Si gel 60 and eluted with $CHCl_3$/MeOH mixtures of increasing polarities. The fractions eluted with 5% MeOH in $CHCl_3$ contained one major compound as indicated by tlc analysis. Further purification of this fraction by gel permeation chromatography over Sephadex (eluent: 50% MeOH in $CHCl_3$) followed by repeated preparative tlc (eluent: 5% MeOH in $CHCl_3$) afforded 4′-*O*-methylstephavanine [**1**] (190 mg).

Dried and powdered roots of *S. abyssinica* were also extracted with cold MeCOEt. Fractionation of this extract following a methodology identical to the one described above afforded stephavanine [**2**] (5 mg).

4′-O-Methylstephavanine [**1**].—Amorphous white solid: mp 187–190° (dec); $[\alpha]^{20}D$ −4° (c=0.01, $CHCl_3$); uv λ max 260, 288 nm; ir ν max 3450, 2950, 1700, 1610, 1520, 1490, 1280, 1230, 1180, 1100 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1; eims *m/z* 511 (68%), 346 (35), 329 (12), 314 (15), 297 (31), 215 (100), 214 (100), 182 (46), 165 (58), 127 (11); hreims *m/z* 511.1829 ($C_{27}H_{29}O_9$ requires 511.1847.

Stephavanine [**2**].—White needles from MeOH: mp 217–220° [lit. (8) 229–230°]; $[\alpha]^{20}D$ −6° (c=0.5, $CHCl_3$); ^{1}H and ^{13}C nmr see Table 1.

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