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THREE ACYCLIC *BIS*-PHENYLPROPANE LIGNANAMIDES FROM FRUITS OF *CANNABIS SATIVA**

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Key Word Index—*Cannabis sativa*; Cannabidaceae; cannabisin E; cannabisin F; cannabisin G.

Abstract—Three new acyclic *bis*-phenylpropane lignanamides, named cannabisin E, F and G were isolated from the fruits of *Cannabis sativa*. Their structures have been elucidated based on spectral and chemical evidence.

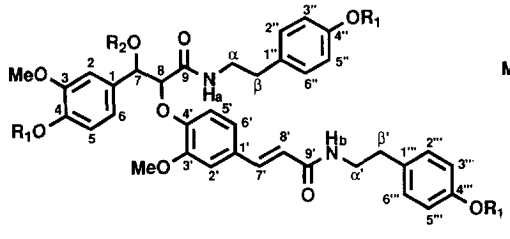
INTRODUCTION

In the course of our investigation, we reported the isolation of cannabisin A–D from the fruits of *C. sativa*. In a continuation of this study, we now report the isolation and identification of three new acyclic *bis*-phenylpropane lignanamides from the same material.

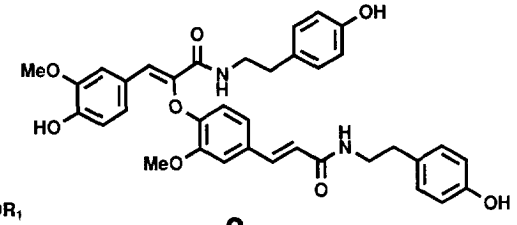
RESULTS AND DISCUSSION

From the *n*-BuOH soluble fraction of the aqueous ethanol extract, cannabisin E (**1**), cannabisin F (**2**) and cannabisin G (**3**) were obtained. Cannabisin E (**1**) was obtained as an amorphous powder. The IR spectrum showed absorption bands for a hydroxyl group (3356 cm^{-1}), an amide group (1658 cm^{-1}) and an aromatic ring (1614 and 1514 cm^{-1}). The molecular formula of **1** was determined to be $C_{36}H_{38}N_2O_9$ by high-resolution FAB mass spectrometry (*m/z*: 643.2234, $[M + H]^+$). Acetylation of **1** gave tetraacetate (**1a**) (δ2.12, 2.27, 2.29 and 2.30) (see Table 1) and methylation of **1** gave pentamethylether (**1b**) (**1** has originally two methoxyls). Thus **1** has three phenolic hydroxyls and an alcohol in its structure.

The NMR spectrum of **1** exhibited two tyramine moieties and two ABX-type coupled aromatic proton signals, along with $-CH(OH)-CH-$ moiety were observed (see Table 1). In the HMBC spectrum of **1**, there were cross peaks between benzylic methine proton signal at H-7 (δ5.17) and C-2 and C-6 aromatic carbon signals (δ111.6 and 120.4), respectively. A cross peak between



1 R_1 R_2 =H
1a R_1 R_2 =Ac
1b R_1=Me R_2=H



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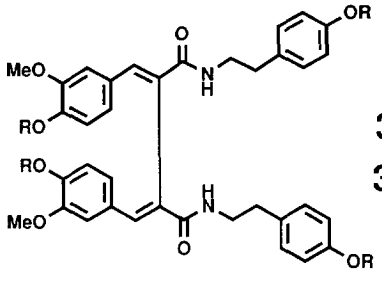

3 R=H
3a R=Ac

*Part 3 in the Series [1, 2].

Table 1. ^{1}H NMR spectral data of **1–3**, **1a**, **1b** and **3a** (500 MHz, acetone-d_6)*

H	1	2	3	1a†	1b‡	3a†
2	7.16 (*d*, 2.0)	7.27 (*d*, 2.0)	7.28 (*d*, 2.1)	6.90 (*d*, 1.9)	7.02 (*d*, 2.0)	7.14 (*d*, 2.0)
5	6.78 (*d*, 8.1)	6.75 (*d*, 8.4)	6.83 (*d*, 8.4)	7.01 (*d*, 8.5)	6.28 (*d*, 8.4)	6.99 (*d*, 8.3)
6	6.94 (*dd*, 8.1, 2.0)	7.03 (*dd*, 8.4, 2.0)	7.08 (*dd*, 8.4, 2.1)	7.06 (*dd*, 8.5, 1.9)	6.90 (*dd*, 8.4, 2.0)	7.09 (*dd*, 8.3, 2.0)
7	5.17 (*brdd*, 6.7, 3.5)	7.27 (*s*)	7.89 (*s*)	6.37 (*d*, 2.5)	5.18 (*d*, 3.1)	8.00 (*s*)
8	4.59 (*d*, 3.5)			4.61 (*d*, 2.5)	4.54 (*d*, 3.1)	
2′	7.14 (*d*, 2.0)	7.35 (*d*, 2.0)	7.28 (*d*, 2.1)	7.09 (*d*, 1.9)	7.11 (*d*, 2.0)	7.14 (*d*, 2.0)
5′	6.53 (*d*, 8.4)	6.78 (*d*, 8.4)	6.83 (*d*, 8.4)	6.02 (*d*, 8.5)	6.89 (*d*, 8.4)	6.99 (*d*, 8.3)
6′	6.96 (*dd*, 8.4, 2.0)	7.14 (*dd*, 8.4, 2.0)	7.08 (*dd*, 8.4, 2.1)	6.87 (*dd*, 8.5, 1.9)	6.98 (*dd*, 8.4, 2.0)	7.09 (*dd*, 8.3, 2.0)
7′	7.42 (*d*, 15.6)	7.46 (*d*, 15.7)	7.89 (*s*)	7.47 (*d*, 15.5)	7.39 (*d*, 15.7)	8.00 (*s*)
8′	6.53 (*d*, 15.6)	6.57 (*d*, 15.7)		6.19 (*d*, 15.5)	6.38 (*d*, 15.7)	
NHa	7.50 (*t*, 6.0)	7.22 (*t*, 6.1)	7.11 (*brt*, 6.5)	7.62 (*t*, 6.1)		6.10 (*t*, 6.1)
NHb	7.21 (*t*, 6.0)	7.25 (*t*, 6.1)	7.11 (*brt*, 6.5)	5.67 (*t*, 6.1)		6.10 (*t*, 6.1)
H-α	3.39 (2H, *ddd*, 14.3, 7.3, 6.0)	3.46 (2H, *dd*, 13.0, 6.0)	3.26 (2H, *dddd*, 13.5, 8.3, 6.5, 2.0)	3.47 (*dt*, 14.0, 6.8) 3.55 (*dt*, 14.0, 6.8)	3.49 (*dd*, 13.4, 6.7) 3.36 (*dd*, 13.4 7.0)	3.33 (2H, *dt*, 13.8, 6.9)
H-α′	3.49 (2H, *ddd*, 13.1, 7.3, 6.0)	3.49 (2H, *dd*, 13.0, 6.0)	3.49 (2H, *dddd*, 13.5, 8.3, 6.5, 2.0)	3.63 (2H, *dd*, 14.0, 6.8)	3.47 (2H, *dd*, 7.4, 8.1)	3.57 (2H, *dt*, 13.8, 6.9)
H-β	2.63 (2H, *dt*, 13.4, 7.3)	2.65 (2H, *t*, 7.1)	2.42 (2H, *ddd*, 13.5, 8.3, 2.0)	2.71 (*dt*, 14.0, 6.8) 2.81 (*dt*, 14.0, 6.8)	2.65 (*dt*, 13.4, 6.7) 2.67 (*dt*, 13.4, 6.7)	2.51 (2H, *dt*, 13.8, 6.9)
H-β′	2.75 (2H, *t*, 7.3)	2.75 (2H, *t*, 7.1)	2.53 (2H, *ddd*, 13.5, 8.3, 2.0)	2.87 (2H, *t*, 6.8)	2.78 (2H, *t*, 7.4)	2.63 (2H, *dt*, 13.8, 6.9)
2″, 6″	6.93 (*d*, 8.6)	6.90 (*d*, 8.6)	6.84 (*d*, 8.6)	7.07 (*d*, 8.6)	6.95 (*d*, 8.8)	6.95 (*d*, 8.6)
3‴, 6‴	7.05 (*d*, 8.6)	7.06 (*d*, 8.6)	6.84 (*d*, 8.6)	7.21 (*d*, 8.6)	7.12 (*d*, 8.8)	6.95 (*d*, 8.6)
3″, 5″	6.70 (*d*, 8.6)	6.68 (*d*, 8.6)	6.68 (*d*, 8.6)	6.91 (*d*, 8.6)	6.69 (*d*, 8.8)	6.90 (*d*, 8.6)
3‴, 6‴	6.75 (*d*, 8.6)	6.75 (*d*, 8.6)	6.68 (*d*, 8.6)	7.03 (*d*, 8.6)	6.82 (*d*, 8.8)	6.90 (*d*, 8.6)
OMe	3.80 (2-OMe) 3.82 (3′-OMe)	3.94 (3-OMe) 3.69 (3′-OMe)	3.74 (3, 3′-OMe)	3.64 (3-OMe) 3.78 (3′-OMe)	3.80 (3-OMe) 3.82 (3′-OMe) 3.74 (4‴-OMe) 3.74 (4-OMe)	3.71 (3,3′-OMe)
OAc				2.12 (7-OAc) 2.27, 2.29, 2.30 (4,4″,4‴-OAc)	3.70 (4″-OMe)	2.27 (4″, 4‴-OAc) 2.28 (4,4′-OAc)

*Coupling constants (Hz) were given in parentheses. Assignments were based on the results of COLOC, HMBC, HMQC, COSY and NOESY spectral data.
†Measured in $CDCl_3$.
‡Measured in $CDCl_3$–MeOH (1:1).

the H-8 proton signal at δ4.59 and quaternary aromatic carbon signal was assignable to C-4′ (δ149.8). On the other hand, in the NOESY spectrum of **1**, there was a cross peak between H-5′ (δ6.53) and the H-8 proton signal. These data supported the presence of an ether function connecting between the C-4′ and C-8. Thus **1** is a surinamensin-type lignan derivative [3], that has a 1-phenyl-2-phenoxyethanol moiety in its structure.

Furthermore, tyramine moieties were assigned on the basis of long-range couplings (HMBC experiment) between the NH proton signal at δ7.21 and carbonylcarbon signals at δ166.2, δ7.50 and δ169.9; those carbonylcarbon signals were assignable to C-9′ and the C-9, respectively (see Tables 1 and 2). The small $J_{7,8}$ values of **1**, **1a** and **1b** [**1** (3.5 Hz), **1a** (2.5 Hz) and **1b** (3.1 Hz)], were indicative of *erythro* derivatives [4–7]. These results led us to conclude the structure of cannabisin E to be **1** (see structure).

Cannabisin F (**2**) was obtained as an amorphous powder. The ^{1}H NMR spectrum was similar to that of **1**, except for the presence of a conjugated olefinic proton (δ7.27) in **2**, instead of $-$CH(OH)$-$CH$-$ function in **1**, and its molecular formula $C_{36}H_{36}N_2O_8$ showed **2** was the dehydrate of **1**. Revealing the presence of an olefine moiety in **2**, **1** was subjected to a dehydration reaction

Table 2. ^{13}C NMR spectral data of **1**–**3**, **1a**, **1b** and **3a** (125 MHz, acetone-d_6)*

C	1	2	3	1a†	1b‡	3a†
1	133.7	125.5	127.8	135.8	134.4	132.6
2	111.6	112.3	113.3	111.4	111.3	113.4
3	148.1	148.3	149.2	151.3	149.6	151.3
4	146.9	147.1	148.3	139.9	149.5	141.3
5	115.2	115.1	116.0	122.9	111.8	123.4
6	120.4	121.5	125.9	119.5	119.9	123.5
7	74.4	123.7	140.5	74.5	74.3	140.1
8	86.5	142.3	127.9	85.4	86.9	128.4
9	169.9	166.1	166.2	168.0	171.2	164.1
1′	131.3	131.7	127.8	130.7	131.1	132.6
2′	112.0	113.6	113.3	111.1	112.0	113.4
3′	151.2	150.2	149.2	149.9	150.8	151.3
4′	149.8	148.8	148.3	148.6	149.8	141.3
5′	117.7	115.9	116.0	117.6	117.4	123.4
6′	121.8	125.5	125.9	121.5	120.5	123.5
7′	139.7	139.6	140.5	140.2	140.8	140.1
8′	121.8	122.0	127.9	120.0	122.2	128.4
9′	166.2	163.3	166.2	165.8	168.2	164.1
α	41.5	41.8	42.6	40.6	41.6	41.3
α′	41.9	42.0	42.6	40.7	42.1	41.3
β	35.5	35.5	35.5	35.0	35.1	34.8
β′	35.7	35.7	35.5	35.0	35.4	34.8
1″	130.8	130.8	130.9	136.4	131.6	135.9
2″	130.5	130.5	130.4	129.6	130.4	129.5
3″	116.1	116.1	116.0	121.6	114.6	121.7
4″	156.7	156.7	156.6	149.3	159.1	149.3
5″	116.1	116.1	116.0	121.6	114.6	121.7
6″	130.5	130.5	130.4	129.6	130.4	129.5
1‴	131.1	131.1	130.9	136.5	132.0	135.9
2‴	130.5	130.5	130.4	129.8	130.4	129.5
3‴	116.1	116.1	116.0	121.8	114.6	121.7
4‴	156.7	156.7	156.6	149.3	159.2	149.3
5‴	116.1	116.1	116.0	121.8	114.6	121.7
6‴	130.5	130.5	130.4	129.8	130.4	129.5
Methoxyl	56.2 (C-3)	56.0 (C-3′)	56.1 (C-3)	55.6 (C-3)	55.6 (C-4)	55.8 (C-3)
	56.3 (C-3′)	56.2 (C-3)	56.1 (C-3′)	56.0 (C-3′)	56.2 (C-3)	55.8 (C-3′)
					56.2 (C-4″)	
					56.3 (C-3′)	
Acetoxyl				20.6 168.8	56.4 (C-4‴)	20.6 168.5
				20.9 169.3		(C-4,4′)
				21.1 169.6		21.1 169.4
				21.1 169.7		(C-4″, 4‴)

*All assignments were based on COLOC, HMBC and HMQC experiments.
†Measured in $CDCl_3$.
‡Measured in $CDCl_3$–MeOH (1:1).

with *p*-toluenesulphonic acid in THF, giving a dehydrant which was identified with cannabisin F. In the COLOC spectrum of **2**, the conjugated olefinic proton (δ 7.27) has two cross peaks with C-2 (δ112.3) and C-6 (δ121.5), respectively. These results showed that the olefine moiety was located at the C-7 and C-8 position. From the above observations, the structure of cannabisin F was concluded as **2** (see structure).

Cannabisin G (**3**) was obtained as an amorphous powder. The ^{1}H NMR spectrum of **3** was similar to that of *N-trans*-feruloyltyramine, except for the presence of a singlet olefinic proton signal (δ7.89), instead of trans olefine proton signals in *N-trans*-feruloyltyramine. The molecular formula of **3** ($C_{36}H_{36}N_2O_8$) showed that **3** was a dimer of *N-trans*-feruloyltyramine. Acetylation of **3** gave tetraacetate **3a** (FAB-MS *m/z*: 793 $[M + H]^+$), supporting its dimeric structure. In the COLOC spectrum of **3a**, there were cross peaks between this singlet olefine proton signal (δ8.00, 2H) and an aromatic methine carbon signal (δ113.4, C-2 and C-2′), another aromatic methine carbon signal (δ123.4, C-6 and C-6′) and a carbonylcarbon signal (δ164.1), respectively. These data suggested that this singlet signal was assignable to the H-7 and the H-7′. Therefore these feruloyl-tyramine units were connected between the C-8 and the C-8′ positions. From these findings, cannabisin G has a biphenylbutadiene moiety and its structure was elucidated as **3** (see structure).

All these cannabisins were presumed to be synthesized during the process of oxidative coupling according to the biosynthesis of lignan [8–10] *N-trans*-feruloyltyramine was subjected to a catalytic condensation with aqueous $FeCl_3$ solution and acetone, affording cannabisin E, F and G as minor products, along with cannabisin D and grossamide as major products. Cannabisin E, F and G are the first naturally occurring acyclic *bis*-phenylpropane lignanamides.

EXPERIMENTAL

Mps: uncorr; ^{1}H and ^{13}C NMR were measured at 500 MHz and 125 MHz, respectively, with TMS as int. standard; 2D NMR: 500 MHz under the same conditions: EI-MS: 70 eV; prep. TLC: prepacked CIG Si-10 column (silica gel, 2.2 cm i.d. × 30 cm); prep. HPLC: YMC R-354 (ODS, 5 cm i.d. × 50 cm): CC: Silica gel 60 (70–230 mesh). Acetylation was conducted with Ac_2O and pyridine. Methylation was conducted by methyliodide with K_2CO_3 and Me_2CO. Plant material was purchased from a Japanese market and a voucher specimen stored in the Herbarium stock room of our Laboratory.

Extraction and isolation. Fruits of *C. sativa* L. (10 kg) were extracted with boiling H_2O–EtOH (1:1) (8 l × 3). The extract was concd to 4 l, which was then extracted with $CHCl_3$ (20 l), and *n*-BuOH (20 l), successively. The *n*-BuOH extract was concd to dryness to give a brown mass (239 g), which was chromatographed on Diaion HP-20 eluted with H_2O (40 l), followed by MeOH (50 l). The MeOH eluate was concd to give a yellow mass (172 g), which was chromatographed on silica gel, eluted with $CHCl_3$–MeOH [4:1 (2 l)], then purified by prep. HPLC [H_2O–MeCN–MeOH (7:2:2)] to give **1** (120 mg), **2** (45 mg) and **3** (20 mg), respectively.

Cannabisin E (**1**). Amorphous powder. FAB-MS *m/z*: 643.2631 ($[M + H]^+$, calcd for $C_{36}H_{39}N_2O_9$; 643.2656). IR ν_{max}^{KBr} cm^{-1}: 3356 (OH), 1658 (C=O), 1614, 1514 (benzene ring). UV λ_{max}^{MeOH} nm (log ε): 226 (4.16), 283 (4.43), 313 (4.31). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2. *Cannabisin F* (**2**). Amorphous powder. FAB-MS *m/z*: 625.2532 ($[M + H]^+$, calcd for $C_{36}H_{37}N_2O_8$; 625.2550). IR ν_{max}^{KBr} cm^{-1}: 3408 (OH), 1658 (C=O), 1614, 1514 (benzene ring). UV λ_{max}^{MeOH} nm (log ε): 287 (4.55), 320 (4.57). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2. *Cannabisin G* (**3**). Amorphous powder. FAB-MS *m/z*: 625.2533 ($[M + H]^+$, calcd for $C_{36}H_{37}N_2O_8$; 625.2550). IR ν_{max}^{KBr} cm^{-1}: 3356 (OH), 1658 (C=O), 1614, 1514 (benzene ring). UV λ_{max}^{MeOH} nm (log ε): 283 (4.48), 316 (4.28). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2. *Cannabisin E trimethyl ether* (**1a**). Amorphous powder. FAB-MS *m/z*: 625.2522 ($[M + H]^+$, calcd for $C_{39}H_{45}N_2O_9$; 685.3125). IR ν_{max}^{KBr} cm^{-1}: 1650, 1614, 1594, 1512. UV λ_{max}^{MeOH} nm (log ε): 225 (4.67), 280 (4.43), 284 (4.45), 314 (4.34). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2. *Cannabisin E tetraacetate* (**1b**). Needles mp 123–125°. FAB-MS *m/z*: 811 ($[M + H]^+$.IR ν_{max}^{KBr} cm^{-1}: 1760, 1658, 1606, 1536, 1218. UV λ_{max}^{MeOH} nm (log ε): 282 (4.37), 290 (4.35), 313 (4.29). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2. *Cannabisin G tetramethyl ether* (**3a**). Amorphous powder. FAB-MS *m/z*: 793 $[M + H]^+$. IR ν_{max}^{KBr} cm^{-1}: 1768, 1642, 1600, 1536, 1194. UV λ_{max}^{MeOH} nm (log ε): 283 (4.44), 316 (4.32). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2.

Dehydration of **1** *with* p-*toluenesulphonic acid.* To a soln of **1** (8.4 mg) with THF (10 ml) was added *p*-toluenesulphonic acid (2 mg). The reaction mixt. was refluxed overnight. The reaction mixt. was diluted with H_2O and then extracted with EtOAc. The EtOAc layer was evapd and then purified by prep. LC [$CHCl_3$–MeOH (1:2, 180 ml)] to give an amorphous powder (5.5 mg), which was identified by direct comparison (^{1}H, ^{13}C NMR, IR, UV) with an authentic sample of cannabisin F (**2**).

Catalytic condensation of N-trans-feruloyltyramine. To a soln of *N-trans*-feruloyltyramine (isolated from this material, 4.13 g) in Me_2CO (25 ml) was added aq. $FeCl_3$ soln (10%, 25 ml) kept at room temp. for 2 days. The reaction mixt. was treated with 2M HCl soln (10 ml), then extracted with EtOAc (100 ml, twice). The EtOAc layer was washed with H_2O and then purified by prep. HPLC (H_2O–MeOH–MeCN, 7:2:2, 4 l) to afford cannabisin D (832 mg), grossamide (535 mg), cannabisin E (22 mg) and cannabisin G (14 mg). Cannabisin F could not be isolated, but was identified by co-TLC and co-injection on analytical HPLC.

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