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CANNABISIN A, AN ARYLNAPHTHALENE LIGNANAMIDE FROM FRUITS OF *CANNABIS SATIVA*

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Key Word Index—*Cannabis sativa*; Cannabidaceae; fruits; cannabisin A; grossamide; *N-trans*-caffeoyltyramine; *N-trans*-feruloyltyramine; *N-p*-coumaroyltyramine.

Abstract—A new lignanamide, named cannabisin A, a known lignanamide, grossamide, and three known amides, *N-trans*-caffeoyltyramine, *N-trans*-feruloyltyramine, *N-p*-coumaroyltyramine, were isolated from the fruits of *Cannabis sativa*. The structure of cannabisin A has been established as 6,7-dihydroxy-1-(3,4-dihydroxyphenyl)-N^1,N^2-*bis*-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalenedicarboxamide, on the basis of chemical and spectral evidence.

INTRODUCTION

Fruits of *Cannabis sativa* have been used as purgatives in China and Japan. Previously, several fatty acids [1], choline and torigonelline [2] were isolated from this material. We have now isolated a new lignanamide, cannabisin A, together with a known lignanamide and three amides.

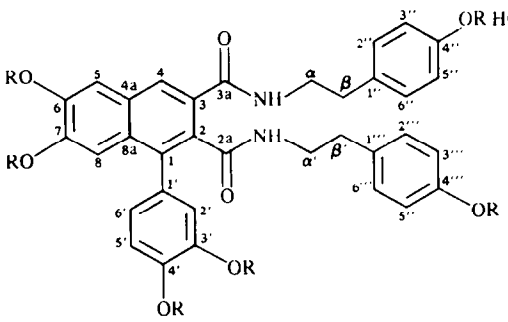
RESULTS AND DISCUSSION

Cannabisin A (**1**) was isolated from an aqueous ethanol extract of *C. sativa*, together with a lignanamide, grossamide (**2**) [3], and three amides, *N-trans*-caffeoyltyramine (**3**), *N-trans*-feruloyltyramine (**4**) and *N-p*-coumaroyltyramine (**5**) [4].

Cannabisin A (**1**) obtained as an amorphous powder, exhibited absorption bands for a hydroxyl group (3368 cm^{-1}), a conjugated amide group (1660 cm^{-1}) and an aromatic ring (1616 and 1580 cm^{-1}) in its IR spectrum. The molecular formula was determined to be $C_{34}H_{30}N_2O_8$ by high-resolution FAB mass spectrometry (*m/z* 595.2132, $[M+H]^+$). The ^1H NMR spectrum of **1** showed signals for two tyramine moieties [δ2.20 (2H, *t*, *J* = 7 Hz), 2.71 (2H, *t*, *J* = 7 Hz), 3.02 (2H, *dt*, *J* = 7, 6 Hz), 3.38 (2H, *dt*, *J* = 7, 6 Hz) (two $-CH_2-CH_2-$systems), 6.65 (2H, *d*, *J* = 8.5 Hz), 6.69 (2H, *d*, *J* = 8.5 Hz), 6.87 (2H, *d*, *J* = 8.5 Hz), 7.05 (2H, *d*, *J* = 8.5 Hz) (two aromatic A_2B_2 systems), 7.66 (1H, *t*, *J* = 6 Hz), 8.06 (1H, *t*, *J* = 6 Hz) (two –NH–)], along with the signals of six aromatic protons [δ6.57 (1H, *dd*, *J* = 8.5, 2.0 Hz), 6.70 (1H, *d*, *J* = 2.0 Hz), 6.81 (1H, *d*, *J* = 8.5 Hz) (ABX system), 6.86 (1H, *s*), 7.18 (1H, *s*), 7.73 (1H, *s*)] from the arylnaphthalene ring.

Acetylation of **1** afforded the hexaacetate (**1a**); FD mass spectrum *m/z* 847 $[M+H]^+$. Acid treatment of **1** with 48% HBr at 120°, followed by methylation, gave **6** as prisms. The UV spectrum of **6** exhibited strong absorption at 257 nm, which was quite similar to that of dehydrodimethylconidendrin (**7**) [5]. The structure of **6** was determined by X-ray crystallographic analysis (crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre) (Fig. 1), which confirmed its arylnaphthalenic structure. The probable reaction mechanism was that both of the tyramine moieties of **1** were hydrolysed followed by elimination of one of the two carboxyl groups. Hence, **1** has an arylnaphthalene skeleton, and one of the tyramine amide groups is located at the C-3 position. In order to confirm the position of the other carbonyl function, **1** was submitted to acid treatment under milder conditions to give **8** as a yellowish powder. The molecular formula of **8** was determined to be $C_{26}H_{19}NO_7$ by high-resolution FAB mass spectrometry (*m/z* 458.1228 $[M+H]^-$). The IR spectrum of **8** showed absorption bands for a five-membered imide ring (1746 and 1690 cm^{-1}). These results suggested that one of the two tyramine moieties of **1** was partially hydrolysed and then formed a five-membered imide ring. Therefore, the two amide carbonyls are oriented *ortho* in relation to one another. Although, biogenetically, they should be at the C-2 and C-3 positions, the possibility that they could be at the C-3 and C-4 positions still remained.

The positions of both the amide carbonyls in **1** were deduced from ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum, the 1,2,4-trisubstituted-patterned aromatic proton signals [δ6.57 (1H, *dd*, *J* = 8.5, 2.0 Hz), 6.70 (1H, *d*, *J* = 2.0 Hz) and 6.81 (1H, *d*, *J* = 8.5 Hz)] were assigned to H-6′, H-2′, H-5′, respectively, and the three aromatic singlet signals at δ6.86, 7.18 and 7.73 to H-5, H-8 and H-2 or 4 on the naphthalene ring. The COLOC [6] spectrum of **1** showed a cross peak between the H-5′ signal and a quaternary aromatic carbon signal at δ129.0, which was only possible to assign to C-1′. This carbon signal showed no cross peak with the naphthalene ring proton signals. On the other hand, the signals of H-2′ and H-6′ showed cross peaks between a quaternary carbon signal at δ135.1, other than C-1′ signal, which also showed a cross peak between the aromatic proton signal at δ6.86, therefore, assignable to H-8. Whilst the remaining two singlet signals at δ7.18 and 7.73 showed cross peaks between aromatic methine carbon signals at δ124.6 and 110.1, respectively, in the COLOC spectrum, direct connections



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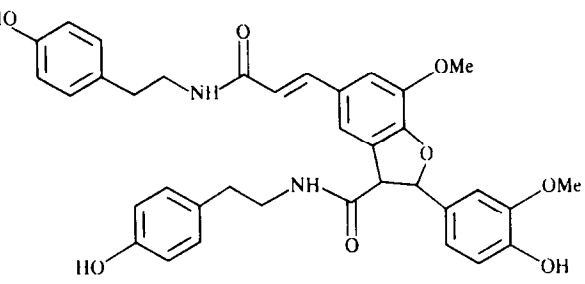
1a R = Ac



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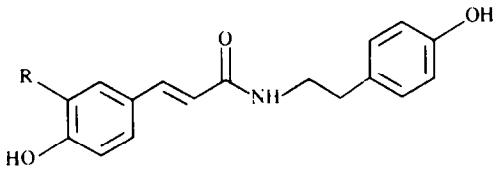
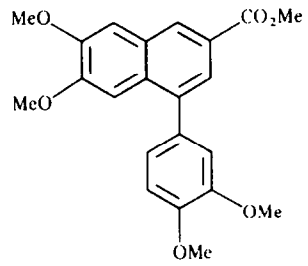


	R
3	OH
4	OMe
5	H



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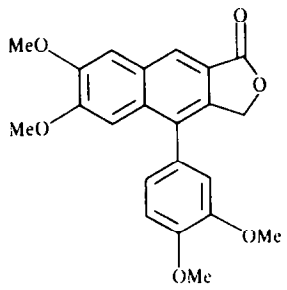

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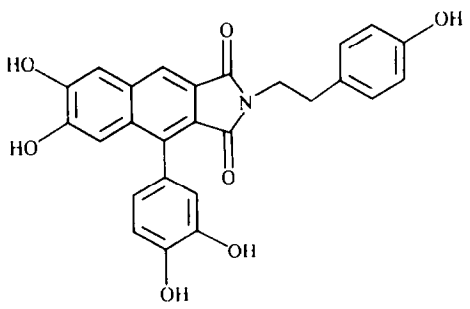

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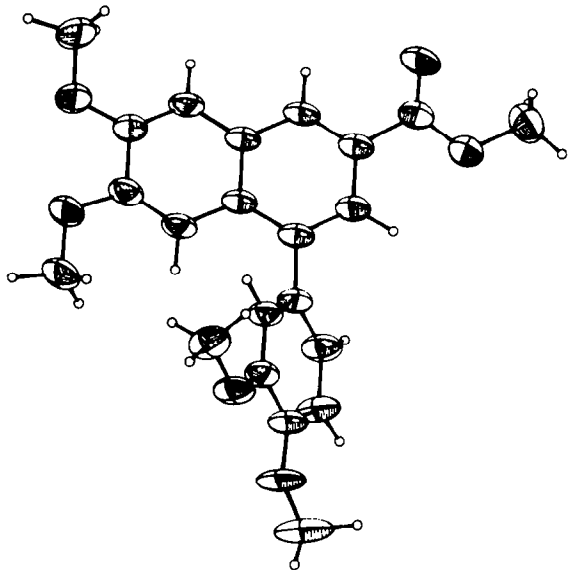

Fig. 1. X-Ray crystallographic analysis of compound **6**.

between δ7.18 and 110.1 and between δ7.73 and 124.6 were observed in the ^1H–^{13}C COSY spectrum. Furthermore, there was a cross peak between δ7.73 and one of the amide carbonyl carbon signal at δ168.5 in the COLOC spectrum. These observations suggested that the δ7.73 signal was assigned to H-4, and accordingly, δ7.18 to H-5. A cross peak between δ7.73 and 7.18 signals in the NOESY spectrum of **1** also confirmed their *peri*-position. The H-4 and the H-5 signal afforded cross peaks with one of NH proton signals at δ8.06, assigned to the NH_a, and also a cross peak with the H-6′ signal and another NH proton signal at δ7.66, assigned to the NH_b proton in the NOESY spectrum. From the X-ray crystallographic data of **6**, it could be shown that the benzene ring twisted to the plane of the naphthalene ring. The tyramine moiety at C-2 was situated above the plane of the benzene ring. Therefore, the proton signals of the tyramine moiety at C-2 appeared upfield to those of C-3 caused by the anisotropic effects of the benzene ring in the ^1H NMR spectrum.

On the basis of the above observations, the positions of the amide carbonyls in **1** were concluded to be at the C-2 and C-3 positions. Thus, the structure of cannabisin A

was elucidated as **1**. Cannabisin A is the first naturally occurring lignanamide with an arylnaphthalene skeleton.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR measured at 500 MHz and 125 MHz, respectively, with TMS as int. std; 2 D NMR: 500 MHz with same conditions. EI-MS: 70 eV, Prep HPLC: prepacked CIG Si-10 column (silica gel, 2.2 cm i.d. × 30 cm). CC: silica gel 60 (70–230 mesh). Acetylation was conducted with Ac_2O and pyridine. Plant material was purchased from a Japanese market and a voucher specimen is stored in the Herbarium stock room of our Institute.

Extraction and isolation. Fruits of *C. sativa* L. (10 kg) were extd 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 ext was concd to dryness to give a brown mass (239.1 g), which was chromatographed on Diaion HP-20, eluting with H_2O (40 l) and then MeOH (50 l). The MeOH eluate was concd to give a yellow mass (171.7 g), which was chromatographed on silica gel, eluting with $CHCl_3$–MeOH. The $CHCl_3$–MeOH (9:1) eluate was purified using a CIG column [$CHCl_3$–MeOH, (9:1)] to give **4** (785 mg) and **5** (1.11 g), respectively. The $CHCl_3$–MeOH (4:1) eluate was concd to remove solvent and then crystallized from MeOH to give **3** (471 mg) as needles. The mother liquor was purified using a CIG column [$CHCl_3$–MeOH, (9:1)] to give **2** (80 mg) as needles from MeOH. The $CHCl_3$–MeOH (1:1) eluate was purified by CC on Sephadex LH-20, eluting with MeOH, to give **1** (740 mg).

Cannabisin A (**1**). Amorphous powder, FAB-MS *m/z*: 595.2130 ($[M+H]^+$, calcd for $C_{34}H_{31}N_2O_8$; 595.2080). IR ν_{max}^{KBr} cm^{-1}: 3368 (OH), 1660 (C=O), 1616, 1580 (benzene ring). UV λ_{max}^{EtOH} nm (log ε): 257 (4.79). ^{1}H NMR (DMSO-d_6): δ2.20 (2H, *t*, *J* = 7 Hz, H-β'), 2.71 (2H, *t*, *J* = 7 Hz, H-β), 3.02 (2H, *dt*, *J* = 7, 6 Hz, H-α'), 3.38 (2H, *dt*, *J* = 7, 6 Hz, H-α), 6.57 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-6'), 6.65 (2H, *d*, *J* = 8.5 Hz, H, -3''', H-5'''), 6.69 (2H, *d*, *J* = 8.5 Hz, H-3'', H-5''), 6.70 (1H, *d*, *J* = 2.0 Hz, H-2'), 6.81 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.86 (1H, *s*, H-8), 6..87 (2H, *d*, *J* = 8.5 Hz, H-2''', H-6'''), 7.05 (2H, *d*, *J* = 8.5 Hz, H-2'', H-6''), 7.18 (1H, *s*, H-5), 7.66 (1H, *t*, *J* = 6 Hz, NH_b), 7.73 (1H, *s*, H-4). 8.06 (1H, *t*, *J* = 6 Hz, NH_a). ^{13}C NMR: see Table 1.

Table 1. ^{13}C NMR spectral data of compounds (125 MHz, DMSO-d_6)*

C†		1		1a		6‡	8
Arylnaphthalene moiety							
1		135.1		134.8		125.4	122.2
2		131.1		134.8		124.9	132.8
3		129.3		133.1		130.3	126.5
4		124.6		126.8		128.6	122.6
5		110.1		122.0		107.8	112.2
6		147.4		141.7		149.9	150.3
7		147.9		142.4		151.4	150.4
8		108.8		118.9		104.8	113.4
4a		127.5		130.0		133.2	128.1
8a		128.4		130.1		129.0	132.9
2a		167.9		167.0		—	169.2
3a		168.5		167.2		167.5	170.0
1'		129.0		134.9		138.9	139.8
2'		117.8		125.2		113.2	116.1
3'		144.4		141.4		148.9	146.4
4'		144.4		141.7		148.6	145.9
5'		114.8		123.2		111.3	118.3
6'		121.3		128.4		122.1	122.9
Tyramine moiety							
1''	1'''	129.5	129.6	136.9	137.0		130.5
2''	2'''	129.4	129.1	129.6	129.3		130.8
3''	3'''	115.0	115.0	121.5	121.4		116.3
4''	4'''	155.5	155.4	148.8	148.7		156.9
5''	5'''	115.0	115.0	121.5	121.5		116.3
6''	6'''	129.4	129.1	129.6	129.4		130.8
α	α'	41.1	40.8	40.7	40.4		40.4
β	β'	34.2	33.8	34.3	33.7		34.5
				acetoxyl		methoxyl	
				20.2	168.0	55.2	
				20.2	168.0	55.9	
				20.3	168.2	55.9	
				20.3	168.2	56.0	
				20.7	169.1	56.2	
				20.8	169.1		

*All assignments were based on ^{13}C–^{1}H COSY and COLOC experiments.
†Carbon numbering system corresponded to ref. [7].
‡In $CDCl_3$; the assignments were referred to ref. [8].

Grossamide (**2**). Needles (MeOH), mp 133–135°. $[\alpha]_D^{25}$ 0° (EtOH; *c* 0.57). FAB-MS *m/z*: 625 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3296, 1656, 1614, 1600. Data identical with those of grossamide [3].

N-trans-*Caffeoyltyramine* (**3**). Needles (MeOH), mp 213–215° [lit. 220–222°]. FD-MS *m/z*: 300 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3336, 1646, 1604, 1582. Identified by direct comparison (mmp, ^1H, ^{13}C NMR, IR and UV) with an authentic sample, prepd as follows. To a soln of caffeic acid (180 mg, 1 mM) in DMF (10 ml) was added tyramine (140 mg, 1 mM) and DEPC (diethylphosphorocyanidate) (0.15 ml); the mixt. was allowed to stand at 0° for 3 hr. The reaction mixt. was washed with H_2O and then extd with EtOAc. The EtOAc layer was evapd to afford caffeoyltyramine (203 mg) as needles from MeOH.

N-trans-*Feruloyltyramine* (**4**). Amorphous powder. EIMS *m/z* (rel. int.): 313 (28, $[M]^+$), 193 (71), 177 (100), 145 (29), 120 (31). IR ν_{max}^{KBr} cm^{-1}: 3328, 1625, 1592, 1514. Identified by direct comparison (^1H, ^{13}C NMR, IR and UV) with an authentic sample prepd as follows. To a soln of ferulic acid (190 mg, 1 mM) in DMF (15 ml) was added tyramine (140 mg, 1 mM) and DEPC (0.15 ml), following by standing at 0° for 3 hr. The reaction mixt. was washed with H_2O and then extd with EtOAc. The EtOAc layer was evapd to afford feruloyltyramine (190 mg) as a powder.

N-p-*Coumaroyltyramine* (**5**). Needles (EtOAc), mp 256–257° [lit. 235–238°]. EI-MS *m/z* (rel. int.): 283 (11, $[M]^+$), 164 (69), 147 (100), 120 (58). IR ν_{max}^{KBr} cm^{-1}: 3316, 1662, 1604, 1536, 1514. Identified by direct comparison (mmp, ^1H, ^{13}C NMR, IR and UV) with an authentic sample which was prepd as follows. To a soln of *p*-coumaric acid (160 mg, 1 mM) in DMF (10 ml) was added tyramine (140 mg, 1 mM) and DEPC (0.15 ml); the mixt. was allowed to stand at 0° for 3 hr. The reaction mixt. was dild with H_2O and then extracted with EtOAc. The EtOAc layer was evapd to afford *p*-coumaroyltyramine (210 mg) as needles from EtOAc.

Cannabisin A hexaacetate (**1a**). Needles ($CHCl_3$), mp 204–207°. FD-MS *m/z*: 847 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 1768, 1640, 1548, 1198. UV λ_{max}^{EtOH} nm (log ε): 244 (4.60), 295 (3.80). ^1H NMR (DMSO-d_6): 2.23, 2.25, 2.28, 2.28, 2.30, 2.35 (each 3H, *s*, OAc × 6), 2.33 (2H, *m*, H-β'), 2.87 (2H, *t*, *J* = 7.5 Hz, H-β), 3.09 (2H, *dt*, *J* = 7.5, 6.3 Hz, H-α'), 3.49 (2H, *dt*, *J* = 7.5, 6.3 Hz, H-α), 6.69 (2H, *d*, *J* = 8.5 Hz, H-3‴, H-5‴), 7.06 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 7.15 (2H, *d*, *J* = 8.5 Hz, H-2‴, H-6‴), 7.22 (1H, *s*, H-8), 7.23 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.24 (1H, *dd*, *J* = 8.8, 2.0 Hz, H-6'), 7.33 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″), 7.38 (1H, *d*, *J* = 8.8 Hz, H-5'), 7.99 (1H, *s*, H-5), 8.09 (1H, *s*, H-4), 8.09 (1H, *t*, *J* = 6 Hz, NH_b), 8.50 (1H, *t*, *J* = 6 Hz, NH_a). ^{13}C NMR see Table 1.

Treatment of compound **1** *with* HBr *at* 120°. To a soln of **1** (350 mg) in pyridine (0.8 ml) was added HBr (48%, 3 ml). The reaction mixt. in a sealed tube was heated at 120° for 3 days. The reaction mixt. was dild with H_2O and then extracted with EtOAc. The EtOAc layer was evapd to give a yellow mass (190 mg), which was methylated with MeI and K_2CO_3 in the usual manner to afford **6** as prisms (52 mg) from Me_2CO, mp 180.6°. EI-MS *m/z* (rel. int.): 382.1416 $[M^+]$ (calcd for $C_{22}H_{22}O_6$: 382.1416) (base). IR ν_{max}^{KBr} cm^{-1}: 1704, 1622, 1510, 1244. UV λ_{max}^{EtOH} nm (log ε): 257 (4.29), 304 (3.69). ^1H NMR ($CDCl_3$): δ3.85 (3H, *s*, CO_2Me), 3.91, 3.96, 3.98, 4.03 (each 3H, *s*, OMe × 4), 7.01 (1H, *d*, *J* = 8.2 Hz, H-5'), 7.05 (1H, *d*, *J* = 1.9 Hz, H-2'), 7.07 (1H, *dd*, *J* = 8.2, 1.9 Hz, H-6'), 7.27 (1H, *s*, H-8), 7.28 (1H, *br s*, H-5), 7.89 (1H, *d*, *J* = 2 Hz, H-2), 8.44 (1H, *d*, *J* = 2 Hz, H-4). ^{13}C NMR see Table 1.

Treatment of **1** *with* HBr *at* 90°. To a soln of **1** (192 mg) in pyridine (2 ml) was added HBr (48%, 3 ml). The reaction mixt. in a sealed tube was heated at 90° for 2 days. The reaction mixt. was dild with H_2O and extracted with EtOAc. The EtOAc layer was evapd to afford **8** as a yellowish powder (114 mg). FAB-MS *m/z*: 458.1228 ($[M+H]^+$, calcd for $C_{26}H_{20}NO_7$; 458.1240). IR ν_{max}^{KBr} cm^{-1}: 3380, 1746, 1690, 1614, 1514. UV λ_{max}^{EtOH} nm (log ε): 289 (4.91). ^1H NMR (DMSO-d_6): δ2.75 (2H, *t*, *J* = 7 Hz, H-β), 3.66 (2H, *dt*, *J* = 7, 6 Hz, H-α), 6.58 (1H, *dd*, *J* = 8.0, 2.1 Hz, H-6'), 6.65 (2H, *d*, *J* = 8.5 Hz, H-3″, 5″), 6.71 (1H, *d*, *J* = 2.1 Hz, H-2'), 6.87 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.97 (2H, *d*, *J* = 8.5 Hz, H-2″, 6″), 7.11 (1H, *d*, *J* = 0.8 Hz, H-8), 7.44 (1H, *s*, H-5), 8.09 (1H, *br d*, *J* = 0.5 Hz, H-4). ^{13}C NMR (DMSO-d_6): see Table 1.

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