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The total synthesis of cannabisin G

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Abstract: A convenient method for the synthesis of lignanamide cannabisin G, starting from vanillin, was developed. The convergent synthesis was based on the Stobbe reaction as C–C bond-forming steps to give the skeleton of lignan, which was condensed with a derivative of tyramine to obtain synthetic cannabisin G for the first time.

Keywords: synthesis; lignanamide; stobbe reaction; cannabisin G.

INTRODUCTION

Cannabisin G (**1**) was first isolated from the fruits of *Cannabis sativa* in 1995.[1] *C. sativa* is an annual plant which belongs to the family Cannabaceae from Central Asia.[2] *C. sativa* has been utilized as an anti-asthma, anticonstipation, anthelminthic drug in traditional Chinese medicine, and these uses are still well-rooted in folk medicine today.[3,4] Cannabinoids, flavonoids, stilbenoids, terpenoids, alkaloids and lignanamides are some of the secondary metabolites present in *C. sativa*.[5] Cannabisin G belongs to the lignanamide group and is classified as lignans of the arylnaphthalene derivative type. Natural products of the lignanamide family displayed interesting and diverse biological activities, including feeding deterrent activity and insecticidal effects.[6,7] In 2002, it was first reported that cannabisin G showed cytotoxic activity against human prostate cancer LNCaP cells.[8]

A synthetic approach to the lignanamide family has not hitherto been reported. Herein, full details of the total synthesis of the lignanamide cannabisin G (**1**) are provided.

In the retrosynthetic analysis (Scheme 1), cannabisin G must be developed for the coupling of (*E*,*E*)-**2** with tyramine. The key intermediate (*E*,*E*)-**2** is obtained by the condensation of vanillin with diethyl succinate.

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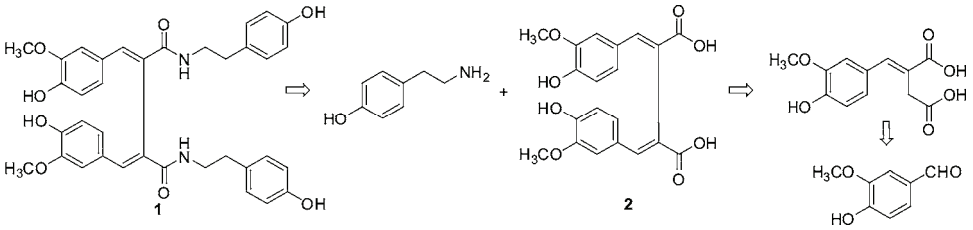
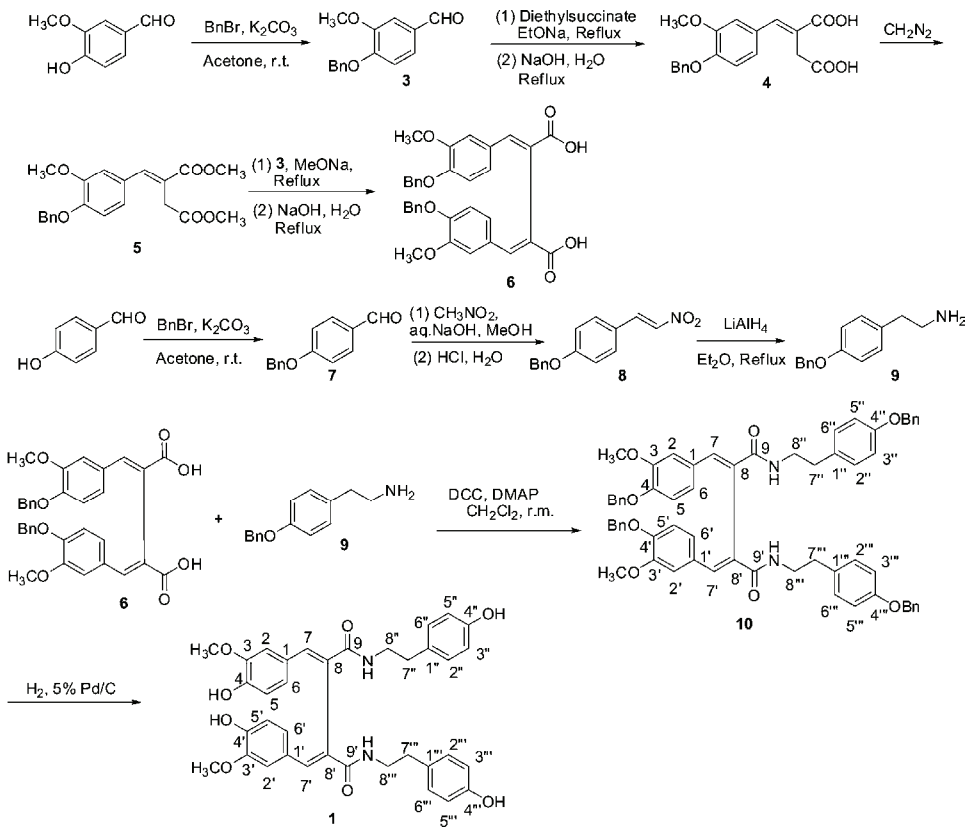


Scheme 1. In the retrosynthetic analysis, cannabisin G must be developed by the coupling of (*E*,*E*)-**2** with tyramine. The key intermediate (*E*,*E*)-**2** was obtained by the condensation of vanillin and diethyl succinate, involving double Stobbe reactions.

As shown in Scheme 2, the synthesis involved the Stobbe reaction to construct the skeleton of lignan (C_6-C_4-C_6), followed by condensation with tyramine to obtain cannabisin G (**1**).



Scheme 2. Reaction scheme for the preparation of cannabisin G starting from vanillin.

EXPERIMENTAL

General

Melting points were taken on a Gallenkamp melting point apparatus and were uncorrected. The infrared spectra were recorded on a Nicolet Nexus 670 FTIR spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AM−500 MHz spectrometer. The mass spectra were recorded on a ZAB−HS spectrometer. HRMS were obtained on a Bruker Daltonics APEXII47e spectrometer. Flash column chromatography was performed on silica gel (200–300 mesh) and TLC inspections on silica gel GF254 plates.

*4-Benzyloxy-3-methoxybenzaldehyde (**3**)*

A mixture of vanillin (60.8 g, 400 mmol), benzyl bromide (68.4 g, 400 mmol) and anhydrous potassium carbonate (55.2 g, 400 mmol) in acetone (200 ml) was stirred overnight at room temperature. The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was crystallized from EtOH to give the compound **3** as yellow crystals (92.0 g).

*(E)-2-(4-benzyloxy-3-methoxybenzylidene)succinic acid (**4**)*

Compound **3** (72.6 g, 300 mmol) and diethyl succinate (52.2 g, 300 mmol) were added to a solution of NaOEt (40.8 g, 600 mmol) in EtOH (500 mL). The mixture was heated under N_2 and was refluxed for 4 h, and then the ethanol was removed. The residue was cooled and acidified with HCl (5 mol L^{-1}, 60 ml). This was then extracted with EtOAc (3×70 mL). The EtOAc layer was then re-extracted with saturated $NaHCO_3$ solution (300 mL). Acidification of the aq. $NaHCO_3$ extract with HCl (5 mol L^{-1}, 60 ml) provided an oily layer, which was again extracted with EtOAc (3×70 mL). The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. This residue was added to a solution of 20 % aqueous NaOH (500 mL) and refluxed for 3 h. After cooling to room temperature, the mixture was washed with EtOAc (3×70 mL). After decolorizing with active carbon, the mixture was acidified with HCl (5 mol L^{-1}, 60 ml) whereby a white solid was obtained. The crude product was crystallized from EtOH to give the diacid **4** as a yellow crystal (120.0 g).

*(E)-Dimethyl 2-(4-benzyloxy-3-methoxybenzylidene)succinate (**5**)*

The diacid **4** (68.4 g, 200 mmol) was added to an ice-cold solution containing an excess of CH_2N_2 in Et_2O. The mixture was stirred for 12 h, and concentrated *in vacuo*. Flash column chromatography of the residue gave diester **5** as a yellow oil (71.8 g).

*(E,E)-2,3-bis(4-benzyloxy-3-methoxybenzylidene)succinic acid (**6**)*

Diester **5** (37.0 g, 100 mmol) on Stobbe condensation (following the above-mentioned procedure) with compound **3** (24.2 g, 100 mmol) provided a light-yellow solid which was purified by recrystallization from MeOH to yield product **6** (42.5 g).

*4-Benzyloxybenzaldehyde (**7**)*

A mixture of 4-hydroxybenzaldehyde (24.4 g, 200 mmol), benzyl bromide (34.0 g, 200 mmol) and anhydrous potassium carbonate (27.6 g, 200 mmol) in acetone(100 ml) were stirred overnight at room temperature. The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was crystallized from EtOH to give the compound **7** as yellow crystals (39.4 g).

*1-Benzyloxy-4-(2-nitroethenyl)benzene (**8**)*

To a mixture of nitromethane (9.2 g, 150 mmol) and compound **7** (31.8 g, 150 mmol) in MeOH (200 mL) was added dropwise sodium hydroxide (8.0 g, 200 mmol) in water (200 mL) under an ice bath. The reaction mixture was stirred for 5 h and then poured into 500 mL of

HCl (2 M) in water. The mixture was filtered and the yellow crystalline mass was washed with water and crystallized from EtOH to give product **8** as yellow crystals (32.1 g).

o-*Benzyltyramine (**9**)*

Compound **8** (12.8 g, 50 mmol) in dry Et_2O was added to the solution of $LiAlH_4$ (5.0 g, 132 mmol) in Et_2O. The mixture was heated at reflux for 4 h under nitrogen. Then the reaction was quenched with ice water and the resulting mixture filtered. The filtrate was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography of the residue gave product **9** (8.8 g).

*Cannabisin G (**1**)*

A solution of compound **6** (2.8 g, 5 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a solution of compound **9** (2.3 g, 10 mmol), *N*,*N*'-dicyclohexylcarbodiimideline, DCC (2.1 g, 10 mmol), and 4-dimethylaminopyridineline, DMAP (1.3 g, 10 mmol), in dry CH_2Cl_2 (100 mL) at 0 °C for 2 h under nitrogen. After stirring the mixture overnight at room temperature, the reaction mixture was filtrated and the solvent was distilled off. Flash column chromatography (petroleumether: ethyl acetate = 6:1) of the residue gave product **10** (4.2 g).

Product **10** (3.0 g, 3.0 mmol) was dissolved in 50 mL MeOH and stirred under a hydrogen atmosphere (1 atm) for 7 h in the presence of 5 % Pd/C (1.5 g). The reaction mixture was filtered through a pad of celite and then the solvent was removed *in vacuo*. Flash column chromatography (petroleumether: ethyl acetate = 5:1) of the residue gave an amorphous powder cannabisin G (**1**) (1.4 g).

RESULTS AND DISCUSSION

The analytic and spectroscopic data of cannabisin G and the intermediate products are given below.

*4-Benzyloxy-3-methoxybenzaldehyde (**3**)*. Yield: 95 %; m.p. 65–67 °C. IR (KBr, cm^{-1}): 3014, 2845, 1679, 1671, 1587, 1505, 1466, 1385, 1276, 1134, 1032. ^{1}H-NMR (500 MHz, DMSO–d_6, δ / ppm): 3.84 (3H, *s*, OCH_3), 5.16 (2H, *s*, $ArCH_2O$), 6.87–7.54 (8H, *m*, ArH), 9.85 (1H, *s*, ArCHO). EI−MS (*m/z*, (%)): 242 (M^+) (12), 214 (7), 151 (2), 91 (100), 67 (13).

*(*E*)-2-(4-Benzyloxy-3-methoxybenzylidene)succinic acid (**4**)*. Yield: 83 %; m.p. 131–133 °C. IR (KBr, cm^{-1}): 3250, 3060, 2912, 1709, 1615, 1497, 1484. ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 3.57 (2H, *s*, CH_2), 3.86 (3H, *s*, OCH_3), 5.15 (2H, *s*, $ArCH_2O$), 6.68–7.43 (8H, *m*, ArH), 7.87 (1H, *s*, ArCH=C). EI−MS (*m/z*, (%)): 342 (M^+) (26), 324 (12), 297 (27), 175 (16), 91 (100).

*(*E*)-Dimethyl 2-(4-benzyloxy-3-methoxybenzylidene)succinate (**5**)*. Yield: 97 %; IR (KBr, cm^{-1}): 3080, 2908, 1712 (CH_2**C**$OOCH_3$), 1641 (**C**$OOCH_3$), 1503, 1465. ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 3.69 (3H, *s*, $COOCH_3$), 3.78 (3H, *s*, $COOCH_3$), 3.83 (3H, *s*, OCH_3), 3.56 (2H, *s*, C**H**$_2COOCH_3$), 5.15 (2H, *s*, $ArCH_2O$), 6.68–7.45 (8H, *m*, ArH), 7.88 (1H, *s*, ArCH=C). EI−MS (*m/z*, (%)): 370 (M^+) (36), 338 (18), 307 (14), 175 (23), 91 (100).

*(*E,E*)-2,3-Bis(4-benzyloxy-3-methoxybenzylidene)succinic acid (**6**)*. Yield: 75 %; m.p. 151–153 °C. IR (KBr, cm^{-1}): 3350, 2900, 1740 (2×**C**$OOCH_3$), 1496, 1241, 1042. ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 3.78 (6H, *s*, 2×OCH_3), 5.16 (4H, *s*,

2×$ArCH_2O$), 6.79 (2H, *d*, ArH, *J* = 8.5 Hz), 7.07 (2H, *dd*, ArH, *J* = 2.0 and 8.5 Hz), 7.19 (2H, *d*, ArH, *J* = 2.0 Hz), 7.28–7.40 (10H, *m*, ArH), 7.96 (2H, *s*, 2×ArCH=C). ^{13}C-NMR ($CDCl_3$, 125 MHz, δ / ppm): 55.7 (2×OCH_3), 70.7 (2×$ArCH_2$), 112.7, 113.1, 123.3, 124.9, 127.2, 127.3, 128.0, 128.6 (2×ArCH=**C**), 136.4 (2×Ar**C**H=C), 144.2, 149.2, 150.1, 172.7 (2×C=O). EI−MS (*m/z*, (%)): 566 (M^+) (2.1), 549 (4.3), 325 (11), 175 (35), 151 (5.2), 91 (100). HRMS Calcd. for $C_{34}H_{31}O_8$ ($M+H^+$): 567.2014, found: 567.2012.

*4-Benzyloxybenzaldehyde (**7**)*. Yield: 93 %; m.p. 72–74 °C. IR (KBr, cm^{-1}): 3034, 2840, 2739, 1690, 1601, 1570, 1510, 1453, 1321, 1262, 1166, 1021. ^{1}H--NMR (500 MHz, $CDCl_3$, δ / ppm): 5.16 (2H, *s*, $ArCH_2O$), 7.05–7.84 (9H, *m*, ArH), 9.88 (1H, *s*, ArCHO). EI−MS (*m/z*, (%)): 212 (M^+) (17), 182 (0.8), 151 (1.8), 121 (1.5), 91 (100), 65 (12).

*1-Benzyloxy-4-(2-nitroethenyl)benzene (**8**)*. Yield: 84 %; m.p. 113–115 °C. IR (KBr, cm^{-1}): 3110, 3042, 2960, 1635, 1607, 1550, 1510, 1346, 1265, 1164. ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 5.15 (2H, *s*, $ArCH_2O$), 7.04 (2H, *d*, ArH, *J* = 8.5 Hz), 7.35–7.50 (7H, *m*, ArH), 7.52 (1H, *d*, ArCH=C**H**, *J* = 13.5 Hz), 7.98 (1H, *d*, Ar**H**=CH, *J* = 13.5 Hz). EI−MS (*m/z*, (%)): 255 (M^+) 1.4), 238 (2.5), 226 (3.2), 151 (2.8), 121 (5.3), 91 (100), 65 (7.1).

o-*Benzyltyramine (**9**)*. Yield: 78 %; m.p. 203–206 °C. IR (KBr, cm^{-1}): 3285, 3050, 3025, 2932, 2867, 2586, 1615, 1597, 1518, 1460, 1259, 1030. ^{1}H--NMR (500 MHz, $CDCl_3$, δ / ppm): 2.82–2.85 (2H, *m*, Ar**H**$_2$$CH_2$), 2.94–2.98 (2H, *m*, $ArCH_2$C**H**$_2$), 5.08 (2H, *s*, $ArCH_2O$), 6.95 (2H, *d*, ArH, *J* = 8.5 Hz), 7.18 (2H, *d*, ArH, *J* = 8.5 Hz), 7.31–7.45 (5H, *m*, ArH). ^{13}C-NMR (125 MHz, $CDCl_3$, δ / ppm): 32.5 (Ar**C**H_2CH_2), 39.8 ($ArCH_2$**C**H_2), 69.6 ($ArCH_2O$), 115.4, 128.1, 128.3, 128.9, 129.9, 130.2, 137.6, 157.6. EI−MS (*m/z*, (%)): 227 (M^+) (8.7), 198 (1.2), 151 (4.9), 121 (23.6), 91 (100); HRMS Calcd. for $C_{15}H_{18}NO$ ($M+H^+$): 228.1383, found: 228.1389.

*4,4',4",4"'-tetrabenzyloxy cannabisin G (**10**)*. Yield: 85 %. ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 2.48 (2H, *dt*, H-7"α, H-7"'α, *J* = 13.5, 7.0 Hz), 2.55 (2H, *dt*, H-7"β, H-7"'β, *J* = 13.5 and 7.0 Hz), 3.28 (2H, *dt*, H-8"α, H-8"'α, *J* = 13.5, 7.0 Hz), 3.52 (2H, *dt*, H-8"β, H-8"'β, *J* = 13.5, 7.0 Hz), 3.78 (6H, *s*, 2×OCH_3), 5.15 (4H, *s*, 2×$ArCH_2$), 5.21 (4H, *s*, 2×$ArCH_2$), 6.51–7.48 (34H, *m*, ArH), 7.96 (2H, *s*, 2×ArCH=C). HRMS Calcd. for $C_{64}H_{64}N_3O_8$ ($M+NH_4^+$): 1002.4688, found: 1002.4683.

*Cannabisin G (**1**)*. Yield: 75 %. IR (KBr, cm^{-1}): 3356, 2910, 1659, 1615, 1517, 1194. ^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 2.43 (2H, *dt*, H-7"α, H-7"'α, *J* = 13.6, 6.5 Hz), 2.51 (2H, *dt*, H-7"β, H-7"'β, *J* = 13.6, 6.5 Hz), 3.25 (2H, *dt*, H--8"α, H-8'"α, *J* = 13.6, 6.5 Hz), 3.50 (2H, *dt*, H-8"β, H-8"'β, *J* = 13.6, 6.5 Hz), 3.75 (6H, *s*, 2×OCH_3), 6.82–7.31 (14H, *m*, ArH), 7.89 (2H, *s*, 2×ArCH=C). ^{13}C--NMR (125 MHz, $CDCl_3$, δ / ppm): 35.6 (C-7", C-7"'), 42.7 (C-8"', C-8"'), 56.8 (2×OCH_3), 113.1, 116.2, 125.4, 127.6, 127.8 (C-8, C-8'), 130.2, 130.7, 140.8 (C-7,

C-7'), 148.3, 149.2, 156.5, 166.8 (C-9, C-9'). EI−MS (*m/z*, (%)): 624 (M^+) (0.2), 339 (18), 337 (9), 151 (23), 91 (100); HRMS Calcd. for $C_{36}H_{40}N_3O_8$ ($M+NH_4^+$): 642.2810, found: 642.2814. The data are consistent with the literature.[1]

As is shown in Scheme 2, vanillin was used as the raw material and the 4-hydroxyl group of vanillin was protected with benzyl chloride to afford product **3**. Compound **3** underwent Stobbe condensation with diethyl succinate in the presence of sodium ethoxide in ethanol to produce compound **4**. The (*E*)-configuration of the olefinic double bond was evident from the appearance of the deshielded vinylic proton at δ 7.87 in its ^{1}H-NMR spectrum.[9] Compound **4** was methylated with diazomethane in diethyl ether to yield the diester **5**. The second Stobbe condensation of **5** with **3** in methanol in the presence of sodium methoxide yielded the key intermediate **6**. The deshielded vinylic proton at δ 7.96 in the ^{1}H-NMR spectrum of **6** indicated the (*E*)-configuration for both olefinic double bonds.[10,11]

4-Hydroxybenzaldehyde was protected with benzyl chloride to give product **7**. Condensation of **7** with nitromethane in the presence of sodium hydroxide gave compound **8**, which was followed by reduction with $LiAlH_4$ to afford intermediate **9**.

The intermediate **6** was condensed with compound **9** in CH_2Cl_2 in the presence of DCC and DMAP, followed by hydrogenolysis with 5 % palladium on charcoal catalyst at room temperature to remove the benzyl group and obtain the target product cannabisin G (**1**). Although it is possible to affect cleavage of the benzyl group in the presence of an olefin, in general, the degree of selectivity is dependent upon the substitution pattern and the level of steric hindrance. Good selectivity was achieved for hydrogenolysis of a benzyl group in the presence of a trisubstituted conjugated olefin.[12]

CONCLUSIONS

In summary, an efficient, high-yielding and convergent synthesis of a lignanamide cannabisin G with an overall yield of 22.3 % was developed. The synthesis was based on the Stobbe reaction for the C–C bond-formation steps to give the skeleton of lignan, which afforded the key intermediate diacid, which was condensed with a derivative of tyramine to obtain the natural product cannabisin G for the first time. The present method is a new avenue for the synthesis of a variety of useful and biologically active lignanamides.

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И З В О Д

ТОТАЛНА СИНТЕЗА КАНАБИСИНА G

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Развијена је погодна синтеза лигнанамида канабисина G, полазећи од ванилина. Конвергентна синтеза заснива се на Стобеовој реакцији, у којој се формира угљеник–угљеник веза скелета лигнана, који је повезан са дериватом тирамина. Овим поступком први пут је добијен синтетички канабисин G.

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