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A SYNTHESIS OF CYTONIN (TAIWANIN A), AN ANTITUMOR LIGNAN

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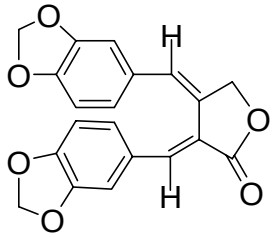
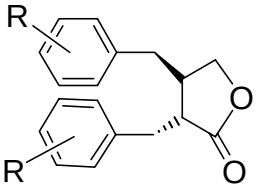
Abstract- A new procedure for the synthesis of the antitumor substance: cytonin (taiwanin A) is described. Stobbe reaction, using diethyl succinate with piperonal in a 2:1 molar ratio, produced 2*E*,3*E*-dipiperonylidenesuccinic acid. Dehydration to the anhydride, followed by reduction with DIBALH gave hydroxy acid. Subsequent cyclization of the hydroxy acid resulted in 21% total yield of cytonin.

Cytonin (originally named taiwanin A) was isolated from the heartwood extracts of *Taiwanin cryptomeriodes Hayata* in Taiwan.[1] Taiwanin A has attracted considerable interest for its biological activities such as herbicidal activity.[2] Lin *et al.* assigned the structure of taiwanin A to be an α,β-bis(piperonylidene)-γ-butyrolactone based on spectral data, and assumed it to have a *2E,3Z* configuration.[3] But, Swoboda *et al.* proposed that the geometry of the diene should be a 2Z,3Z configuration by reasoning of the severe steric strain between the two aryl groups.[4] However, a 2*E*,3*E* stereochemistry was proposed and supported by Hart *et al.*, using X-Ray crystallographic and NMR analysis,[5] and later confirmed by Sai *et al.*, using a cross coupling reaction to synthesize all isomers of taiwanin A, leading to a clear determination of its stereochemistry.[6] Recently taiwanin A has been found to possess similar antitumor activity with taxol by interacting with the microtubilin in the tumor cell, and was named "cytonin" under clinic trial application.[7]



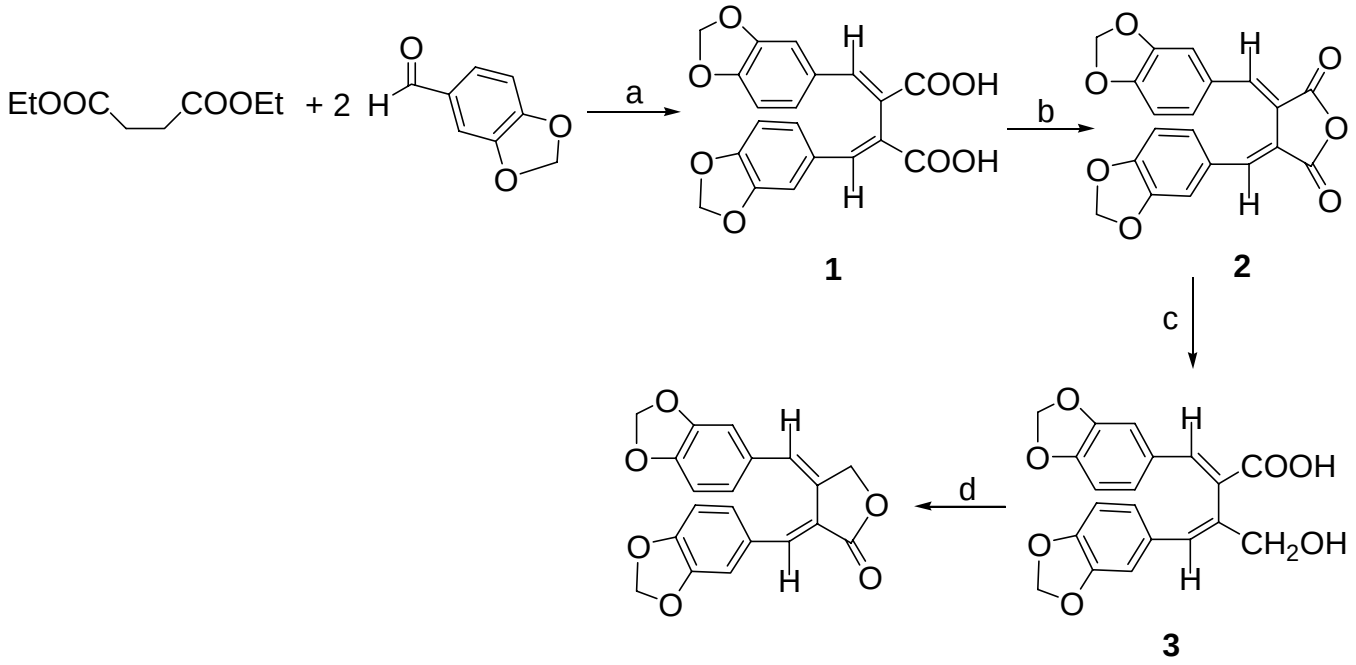
cytonin(taiwanin A)



2,3-disubstituted 4-butanolide

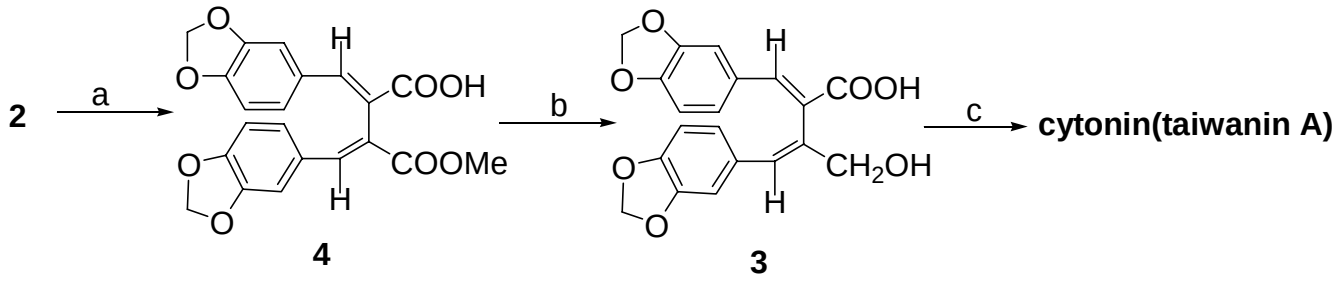
The skeleton of 2,3-disubstituted 4-butanolide is the most important feature found in lignans. Natural antitumor lignans have been recognized as challenging targets for organic synthesis.[8] We are interested in developing a simple synthetic process for commercial large scale preparation of cytonin, to be used for pharmacokinetic studies. Cytonin was synthesized by modification of a known procedure[4] as shown in Scheme 1. Stobbe condensation was carried out by refluxing piperonal (2 eq.) with diethyl succinate (1 eq.) in the presence of potassium (2.2 eq.) in dry *tert*-BuOH for 3 h to give the mixture of isomeric α,β-dipiperonylidenesuccinic acids. It was fortunate that we could obtain pure 2*E*,3*E* isomer **(1)** in 50% yield after neutralization (1N HCl), extraction (ether) and recrystallization with ethyl acetate/hexane. No chromatography was required. Lower yield (< 40%) was observed if condensation was carried out with MeONa or EtONa in dry MeOH or EtOH. The diacid **(1)** was dehydrated with Ac_2O in THF under reflux

for 2 h to give 2*E*,3*E*-dipiperonylidenesuccinic anhydride **(2)** in 85% yield. The anhydride **(2)** was reduced with diisobutylaluminum hydride to provide **(3)**,[4] and subsequent cyclization of **3**, using methyl chloroformate and Et_3N provided the desired cytonin in 50% yield. The physical properties and biological activities of synthetic cytonin were confirmed to be identical with those of the natural molecule.



Scheme 1 *Reagents and conditions*: (a) *tert*-BuOK, *tert*-BuOH; (b) Ac_2O, THF, reflux; (c) DIBALH, CH_2Cl_2, 0 ^oC (d) Et_3N, $ClCO_2Me$, EtOAc.

The second approach followed the procedure of Swoboda *et al.*[4] (Scheme 2). The anhydride **(2)** was treated with MeOH, Et_3N and 4-DMAP to afford methyl hydrogen 2*E*,3*E*-dipiperonylidenesuccinate **(4)** in 96% yield. Selective reduction of the methyl ester group of **4** with $LiBH_4$ in THF provided 2*E*,3*E*-dipiperonylidene-4-hydroxybutyric acid **(3)**, and subsequent cyclization using methyl chloroformate, Et_3N gave cytonin only in 8% yield after column chromatography. When the reduction was carried out in ether, cytonin was obtained in relatively high yield (15%). The low yield resulted from many spots being generated when compound **(4)** was reduced by $LiBH_4$ (followed by TLC).



Scheme 2 *Reagents and conditions*: (a) Et_3N, MeOH, 4-DMAP; (b) $LiBH_4$, ether, reflux; (c) Et_3N, $ClCO_2Me$, EtOAc.

The advantage of the first approach, as compared to the Swoboda *et al.* report,[4] was that: i) no chromatography was required, ii) The reaction of the Stobbe condensation in one pot reduced reaction time from one week (-10 ^oC, Swoboda *et al.*)[4] to 3 h (reflux), and elevated the total yield from 6% to 21%, iii) total synthesis required only 3 days using just four simple steps.

In summary, we explored a simple condition for commercial scale preparation of cytonin. This method is

simpler than the complex two-step Stobbe condensation reported by Momose,[9] and is more efficient than the comprehensive synthetic method using six steps synthesis method as reported by Sai.[6]

EXPERIMENTAL

Column chromatography was performed on silica gel 60 (70-230 mesh, 230-400 mesh, Merck). *tert*-BuOH, ether, and CH_2Cl_2 were dried by CaH_2, THF was dried by Na and distilled. The ^1H NMR spectra were recorded on a Bruker ASPECT 3000 (400 MHz). The value of δ is expressed in ppm, relative to the solvent signal as internal standard ($CHCl_3$, 7.24 ppm; DMSO, 2.49 ppm). MS spectra were measured with Finnigan LCQ. Elemental analyses were performed by Perkin-Elmer2400-CHN. All reagents were commercially available.

(2*E*,3*E*)-2.3-Bis(3,4-methylenedioxybenzylidene)succinic acid (1):[4] To a solution of freshly distilled piperonal (16.5 g, 110 mmol, bp: 89 ^oC/0.2 torr) and diethyl succinate (8.71 g, 50.0 mmol) in dry *tert*-BuOH (100 mL), a solution of *tert*-BuOK (prepared from 4.18 g of potassium, 110 mmol) in *tert*-BuOH (200 mL) was added , and the mixture was heated to reflux for 3 h. The resulting solution was diluted with ether (150 mL) and filtrated to give a yellow solid. The yellow solid was treated with HCl (1N, 200 mL) and extracted with ether (300 mL). The organic layer was washed with water (300 mL) and dried over $MgSO_4$ and concentrated *in vacuo*. To the residue was added ethyl acetate/hexane (1:1, 100 mL) and the crystals were filtrated to give 2*E*,3*E*-dipiperonylidenesuccinic acid **(1)** (8.50 g) as a yellow solid. The filtrate was concentrated *in vacuo* and the residue was treated with ethyl acetate/hexane (1:1, 40 mL). The solution was kept at -10 ^oC for 6 h. The resulting precipitate was filtrated to give **1** (1.00 g). Product **(1)** was obtained in 50% yield (9.5 g, based on diethyl succinate). **1**: yellow solid; mp: 218-220 ^oC (crystallized with ethyl acetate/hexane = 1/1). ^1H NMR (400 MHz, DMSO-d_6) δ 7.40 (s, 2H, vinyl CH), 7.11 (d, *J* = 1.4 Hz, 2H, ArH), 7.03 (dd, *J* = 8.1, 1.4 Hz, 2H, ArH), 6.85 (d, *J* = 8.1 Hz, 2H, ArH), 5.97 (s, 4H, OCH_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.6,148.5, 147.6, 140.2, 128.6, 126.1, 125.7, 105.6, 108.1, 101.5; Electrospray-MS: m/z (%) = 382.8 (M^++1, 63), 365.1 (100), 337 (24).

(2*E*,3*E*)-2,3-Bis(3,4-methylenedioxybenzylidene)succinic anhydride (2):[4] The solution of 2*E*,3*E*-dipiperonylidene succinic acid **(1)** (14.7 g, 38.6 mmol) and Ac_2O (5.43 mL, 57.9 mmol) in THF (50 mL) was refluxed for 2 h and kept at rt for 1 h. The resulting solution was concentrated *in vacuo* and the residue was treated with a mixture of ethyl acetate/hexane (2:3, 200 mL). The precipitate was filtrated to give the 2*E*,3*E*-dipiperonylidenesuccinic anhydride **(2)** (11.9 g, 85%). **2**: orange solid; mp: 210-212 ^oC (crystallized with ethyl acetate/hexane = 2/3). ^1H NMR (400 MHz, DMSO-d_6) δ 7.73 (s, 2H, vinyl CH), 6.72 (d, *J* = 8.1 Hz, 2H, ArH), 6.60 (d, *J* = 8.1 Hz, 2H, ArH), 6.29 (s, 2H, ArH), 5.93 (s, 4H, OCH_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 149.8, 146.0, 137.1, 128.6, 126.3, 118.6, 109.0, 107.0, 101.6; Electrospray-MS: m/z (%) = 364.1 (M^++1, 36), 359.2 (100), 331.2 (36).

Methyl hydrogen (2*E*,3*E*)-2,3-bis(3,4-methylenedioxybenzylidene)succinate (4): The solution of anhydride **(2)** (17.2 g, 47.0 mmol), 4-DMAP (0.57 g, 4.67 mmol) and Et_3N (13.1 mL, 94 mmol) in dry MeOH (200 mL) was heated to reflux for 2 h, and then cooled to rt. The resulting solution was concentrated *in vacuo* and acidified with 1N aqueous HCl (200 mL). The resulting mixture was extracted with ethyl acetate (200 mL), and the organic layer was washed with H_2O (100 mL) followed by drying ($MgSO_4$) and concentration *in vacuo*. The residue was treated with ether/hexane (1:1, 100 mL) and the resulting precipitate was filtrated to give the desired product **(4)** as a pale yellow solid; mp: 180-181 ^oC (crystallized with ether/hexane = 1/1). ^1H NMR (400 MHz, DMSO-d_6) δ 7.74, 7.73 (s, 2H, 2 vinyl CH), 7.20-7.00 (m, 4H, ArH), 6.95-6.80 (m, 2H, ArH), 6.01, 6.00 (s, 4H, 2 OCH_2O). 3.65 (s, 3H, OMe) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ167.5, 166.8, 148.8, 148.7, 147.6, 140.9, 140.7, 128.4, 128.3, 126.1, 125.7, 125.3, 124.8, 108.6, 108.2, 108.1, 101.6, 101.6, 52.2. Anal. Calcd for $C_{21}H_{16}O_8$: C 63.64, H 4.07. Found:

C 63.44, H 4.18.

(2*E*,3*E*)-2,3-Bis(3,4-methylenedioxybenzylidene)-γ-butyrolactone (Cytonin):[4,6]
Method A. The solution of 2*E*,3*E*-dipiperonylidenesuccinic anhydride **(2)** (6.5 g, 18 mmol) in dry CH_2Cl_2 (60 mL) was stirred at 0 ^oC for 15 min. DIBALH (20% in toluene, 45 mL, 54 mmol) was added to the solution slowly and the solution was stirred at 0 ^oC for further 1 h. The resulting solution was acidified with aqueous 10% citric acid (100 mL) and extracted by ethyl acetate. The organic layer was washed with water (100 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product **(3)**. Without further purification, the crude product was dissolved in ethyl acetate (60 mL). Methyl chlorofomate (2 mL) was added to the solution at 0 ^oC and the solution was stirred at 0 ^oC for 20 min. Et_3N (5 mL) was added to the solution very slowly and the solution was stirred at 0 ^oC for 40 min. The resulting solution was treated with ethyl acetate (100 mL), and washed successively with aqueous 10% citric acid (100 mL) and water (100 mL). The organic layer was concentrated *in vacuo* and the residue was crystallized with methanol/hexane (4:1, 200 mL). Filtration of the crystals gave cytonin (3.15 g, 50%) as a yellow solid mp: 198-199 ^oC. ^1H NMR (400 MHz, DMSO-d_6) δ 7.45 (s, 1H, vinyl CH), 6.74 (dd, *J* = 8.0, 1.3, Hz, 1H, ArH), 6.66 (s, 1H, vinyl CH), 6.62 (d, *J* = 8.0 Hz, 1H, ArH), 6.53 (d, *J* = 8.0 Hz, 1H, ArH), 6.42 (dd, *J* = 8.0, 1.3 Hz, 1H, ArH), 6.34 (d, *J* = 1.3 Hz, 1H, ArH), 6.15 (d, *J* = 1.3 Hz, 1H, ArH), 5.89 (s, 2H, OCH_2O), 5.82 (s, 2H, OCH_2O), 5.03 (d, *J* = 1.6 Hz, 2H, CH_2), ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 149.5, 147.6, 146.4, 146.3, 136.6, 130.9, 129.5, 128.3, 125.9, 125.4, 122.7, 119.5, 108.9, 107.9, 107.0, 101.3, 101.0, 71.1; Electrospray-MS: m/z (%) = 351.1 (M^++1, 100).

Method B. To a solution of $LiBH_4$ (0.15 g, 6.80 mmol) and $B(OMe)_3$ (0.02 mL, 0.17 mmol) in ether (50 mL) was added methyl hydrogen 2*E*,3*E*-dipiperonylidene succinate **(4)** (0.68 g, 1.70 mmol). The resulting mixture was refluxed for 5 h. 1N aqueous HCl (50 mL) was added and the solution was extracted with ether. The ether phase was washed with a 10% $NaHCO_3$ solution, and the aqueous solution was acidified with aqueous 10% citric acid to give the crude product **(3)**. The crude product **(3)** was cyclised by the same procedure as method A (see above) to give cytonin (90 mg, 15%) after column chromatography (eluted with hexane/ ethyl acetate 2:1).

ACKNOWLEDGEMENT

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