

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

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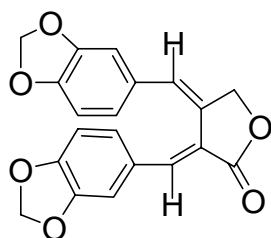
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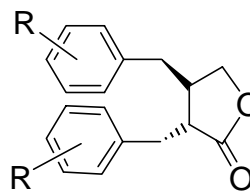
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Abstract- A new procedure for the synthesis of the antitumor substance: cytonin (taiwanin A) is described. Stobbe reaction, using diethyl succinate with piperonal in a 2:1 molar ratio, produced *2E,3E*-dipiperonylidenesuccinic acid. Dehydration to 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 *2E,3E* 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 microtubulin in the tumor cell, and was named "cytonin" under clinic trial application.⁷



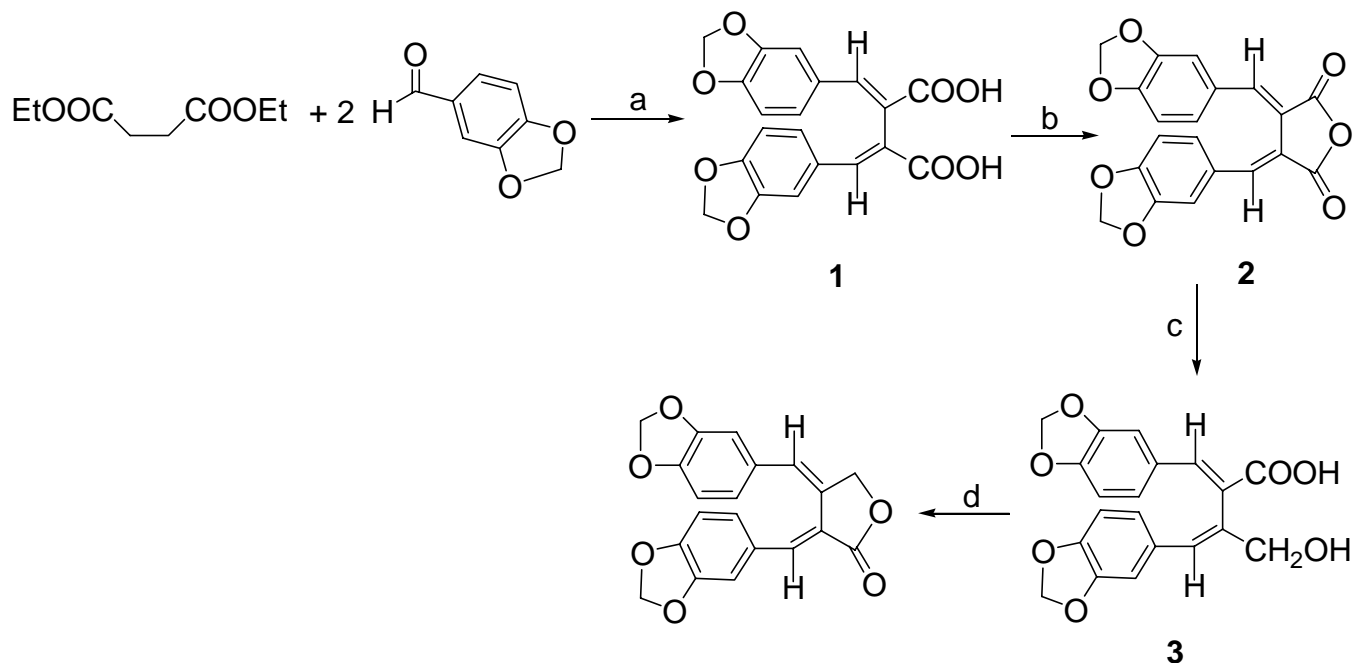
cytonin(taiwanin A)



2,3-disubstituted 4-butanolide

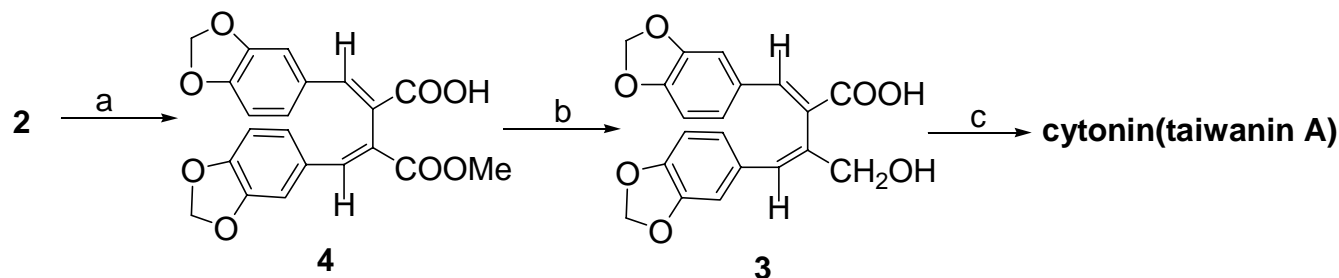
The skeleton of 2,3-disubstituted 4-butanolide is the most important feature found in lignans. Natural antitumor lignans have been recognized as challenging targets for organic synthesis.⁸ We are interested in developing a simple synthetic process for commercial large scale preparation of cytonin, to be used for pharmacokinetic studies. Cytonin was synthesized by modification of a known procedure⁴ 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 *2E,3E* 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 Ac₂O in THF under reflux

for 2 h to give *2E,3E*-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 Et₃N 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) Ac₂O, THF, reflux; (c) DIBALH, CH₂Cl₂, 0 °C (d) Et₃N, ClCO₂Me, EtOAc.

The second approach followed the procedure of Swoboda *et al.*⁴ (Scheme 2). The anhydride (**2**) was treated with MeOH, Et₃N and 4-DMAP to afford methyl hydrogen *2E,3E*-dipiperonylidenesuccinate (**4**) in 96% yield. Selective reduction of the methyl ester group of **4** with LiBH₄ in THF provided *2E,3E*-dipiperonylidene-4-hydroxybutyric acid (**3**), and subsequent cyclization using methyl chloroformate, Et₃N 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 LiBH₄ (followed by TLC).



Scheme 2 Reagents and conditions: (a) Et₃N, MeOH, 4-DMAP; (b) LiBH₄, ether, reflux; (c) Et₃N, ClCO₂Me, 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 °C, 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 CH₂Cl₂ were dried by CaH₂, 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 (CHCl₃, 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 °C/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 MgSO₄ 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*-dipiperonylidene succinic 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 °C 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 °C (crystallized with ethyl acetate/hexane = 1/1). ¹H NMR (400 MHz, DMSO-d₆) 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, OCH₂O); ¹³C NMR (100 MHz, DMSO-d₆) 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 Ac₂O (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*-dipiperonylidene succinic anhydride (**2**) (11.9 g, 85%). **2**: orange solid; mp: 210-212 °C (crystallized with ethyl acetate/hexane = 2/3). ¹H NMR (400 MHz, DMSO-d₆) 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, OCH₂O); ¹³C NMR (100 MHz, DMSO-d₆) 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 Et₃N (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 H₂O (100 mL) followed by drying (MgSO₄) 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 °C (crystallized with ether/hexane = 1/1). ¹H NMR (400 MHz, DMSO-d₆) 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 OCH₂O). 3.65 (s, 3H, OMe); ¹³C NMR (100 MHz, DMSO-d₆) δ 167.5, 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 C₂₁H₁₆O₈: C 63.64, H 4.07. Found:

C 63.44, H 4.18.

(2E,3E)-2,3-Bis(3,4-methylenedioxybenzylidene)- γ -butyrolactone (Cytonin):^{4,6}

Method A. The solution of 2E,3E-dipiperonylidene succinic anhydride (**2**) (6.5 g, 18 mmol) in dry CH₂Cl₂ (60 mL) was stirred at 0 °C for 15 min. DIBALH (20% in toluene, 45 mL, 54 mmol) was added to the solution slowly and the solution was stirred at 0 °C 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 (MgSO₄) and concentrated *in vacuo* to give the crude product (**3**). Without further purification, the crude product was dissolved in ethyl acetate (60 mL). Methyl chloroformate (2 mL) was added to the solution at 0 °C and the solution was stirred at 0 °C for 20 min. Et₃N (5 mL) was added to the solution very slowly and the solution was stirred at 0 °C 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 °C. ¹H NMR (400 MHz, DMSO-d₆) 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, OCH₂O), 5.82 (s, 2H, OCH₂O), 5.03 (d, *J* = 1.6 Hz, 2H, CH₂), ¹³C NMR (100 MHz, CDCl₃) 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; Electropray-MS: *m/z* (%) = 351.1 (M⁺+1, 100).

Method B. To a solution of LiBH₄ (0.15 g, 6.80 mmol) and B(OMe)₃ (0.02 mL, 0.17 mmol) in ether (50 mL) was added methyl hydrogen 2E,3E-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% NaHCO₃ 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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