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Synthesis of *N*-formylnornuciferine with Cardiotonic Activity

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ABSTRACT

(-)-*N*-Formylnornuciferine (1), an aporphine alkaloid isolated from stems of *Tinospora crispa* (*Borapet* in Thai), showed *in vitro* cardiotonic activity. In an earlier study, it exhibited a significant increase in the force of contraction on the atria of an isolated rat heart with no significant change in the heart rate. As only very limited amounts of this active compound can be isolated from *T. crispa*, the evaluation of cardiotonic activity is hampered. One way to obtain sufficient quantities for pharmacological investigation is by chemical synthesis. In the current study, (±)-*N*-formylnornuciferine (1a) was successfully synthesized in six steps from homoveratrylamine with an overall yield of 50%. A palladium-catalyzed coupling reaction was the key step in this synthesis. In the subsequent cardiotonic activity test, it was found that the racemic mixture, (±)-*N*-formylnornuciferine (1a), produced a reduction in the force of contraction and the heart rate, which was different from the activity of the natural aporphine alkoloid isolated from *T. crispa*.

Key words: *N*-formylnornuciferine, synthesis, cardiotonic activity, *Tinospora crispa*, borapet

INTRODUCTION

Aporphines are a large group of alkaloids with more than 500 structures reported to date. The general structure and numbering system of aporphine alkaloids are shown in Figure 1.

Aporphine alkaloids are widely distributed in many plant species, of genuses including *Annonaceae, Lauraceae, Monimiaceae, Menispermaceae, Hernandiaceae, and Ranunculaceae*. Many aporphines demonstrate various biological activities, such as antioxidant, antiplatelet, antitumor, anticonvulsant, antiplasmodial, antineoplastic, antimalarial, antiprotozoal, antipoliovirus, cytotoxic, and antiparkinsonian effects (Zhang *et al*., 2007).

(-)-*N*-Formylnornuciferine (1) is one of the naturally occurring aporphine alkaloid types (Figure 2). It has been isolated from many plants, including *Guyanese annonaceous* (Cortes *et al*.,

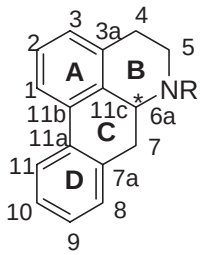

Figure 1 General structure and numbering system of aporphine alkaloids.

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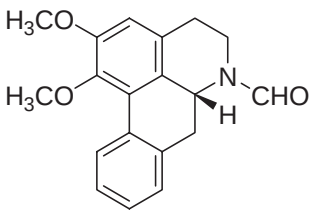

Figure 2 Structure of (-)-*N*-formylnornuciferine (1).

1986), *Tinospora crispa* (Pachaly *et al*., 1992; Cavin *et al*., 1998), *Piptostigma fugax* (Achenbach and Schwinn, 1995) and *Piper argyrophylum* (Simonsen *et al*., 1996). In 2007, a research group involving the authors isolated a pure aporphine alkaloid, (-)-*N*-formylnornuciferine (1), from the ethanol extracts of *T. crispa* (Kongkathip *et al*., 2007).

(-)-*N*-Formylnornuciferine (1) showed *in vitro* cardiotonic activity. It exhibited a significant increase in the force of contraction of an isolated rat heart with no significant change in the heart rate (Sunthikawinsakul, 2005). Unfortunately, this alkaloid was isolated from *T. crispa* in very small quantities. The evaluation of this active alkaloid is hampered by a lack of material. At present, one way to obtain sufficient quantities for pharmacological investigation is by chemical synthesis.

MATERIALS AND METHODS

Reactions were monitored by TLC on aluminum sheets SIL G/UV254 from Merck. Melting points were determined on an electrothermal SMP-10 apparatus and were uncorrected. Infrared spectra were recorded on a Perkin–Elmer 2000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 300 spectrophotometer operating at 400 MHz (proton) and 100 MHz (carbon-13), with the chloroform peak used as a standard. Chemical shifts were expressed in parts per million (ppm) and Hz respectively. Mass spectra were obtained on an Agilent Technology 1100 series LL/MSD Trap, where the first number denotes the *m/z* value and the ion assignment and abundance are given in parentheses. All analytical grade chemicals and solvents were purchased from Fluka Co. Ltd. and solvents were purified by general methods before being used.

Synthetic procedures

N-(3,4-Dimethoxyphenethyl)-2-(2-bromophenyl)acetamide (5)

A solution of 2-bromophenylacetic acid (2) (643 mg, 2.99 mmol), oxalyl chloride (0.6 mL, 4.48 mmol) and a catalytic amount of dimethylformamide (3 drops) in benzene (6 mL) was stirred at room temperature. After 2 h, the solvent was removed under reduced pressure to give bromophenylacetyl chloride (3) which was used in the next step without further purification. The resulting acid chloride (3) in dichloromethane (3 mL) was added to a mixture of homoveratrylamine (4) (500 mg, 2.99 mmol) and sodium carbonate (380 mg, 3.59 mmol) in dichloromethane (3 mL) and water (3 mL). The reaction mixture was stirred at room temperature for 2 h. Layers were separated and the aqueous layer was extracted with dichloromethane (3×50 mL). The combined organic phase was washed with water, brined and then dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was recrystallized from ethanol to give *N*-(3,4-dimethoxyphenethyl)-2-(2-bromophenyl)acetamide (5) (1.03 g, 91%) as a white needle crystal, m.p. 126 to 128°C. **FTIR** (KBr), υ_{max}, 3309, 1647, 1546 cm^{-1}. **^{1}H NMR** ($CDCl_3$): 7.46 (d, *J*=8.2, 1H, H-3'), 7.20-7.18 (m, 2H, H-6', H-4'), 7.08-7.04 (m, 1H, H-5'), 6.64 (d, *J*=8.1 Hz, 1H, H-5), 6.56 (d, *J*=2.0 Hz, 1H, H-2), 6.52 (dd, *J*=8.1, 2.0 Hz, 1H, H-6), 3.77 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.58 (s, 2H, $ArC\underline{H}_2CO$), 3.39 (dd, *J*=12.8, 6.9 Hz, 2H, $ArCH_2C\underline{H}_2NH_2$), 2.63 (t, *J*=6.9 Hz, 2H, $ArC\underline{H}_2CH_2NH_2$). **^{13}C NMR** ($CDCl_3$): 169.4

(C=O), 149.0, 147.6 (C-3, C-4), 134.7 (C-2'), 133.0 (C-3'), 131.6 (C-4'), 131.0 (C-1'), 129.0 (C-5'), 127.9 (C-6'), 124.9 (C-1), 120.5 (C-6), 111.8 (C-2), 111.3 (C-5), 55.9 (OCH_3), 55.8 (OCH_3), 44.0 ($ArCH_2CO$), 40.7 ($ArCH_2CH_2NH_2$), 34.9 ($ArCH_2CH_2NH_2$). **MS** (EI), *m/z* (relative intensity): 377 (8), 164 (100), 151 (11).

1-(2-Bromobenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline (6)

Phosphorus oxychloride (0.48 mL, 5.29 mmol) was added dropwise into a cool solution of *N*-(3,4-dimethoxyphenethyl)-2-(2-bromophenyl) acetamide (5) (500 mg, 1.32 mmol) in dry dichloromethane (6 mL). The reaction mixture was refluxed under nitrogen atmosphere for 4 h. After cooling to 0°C, to a cool solution was carefully added 10% sodium hydroxide and then extracted with dichloromethane (3×100 mL). The combined organic layer was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to produce the crude product of 1-(2-bromobenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline (6), which was used in the next step without further purification.

1-(2-Bromobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (7)

Sodium borohydride (65 mg, 1.71 mmol) was slowly added to a cool solution (0°C) of crude 1-(2-bromobenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline (6) in methanol (6 mL) and stirred at room temperature for 2 h. Brine was added to a cool reaction mixture and extracted with dichloromethane (3×50 ml). The organic layer was separated and dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure. The residue was purified by flash column chromatography using methanol-dichloromethane (3:97) to give 1-(2-bromobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (7) (0.39 g, 83% (two steps)) as a yellow liquid. **FTIR** (neat), υ_{max}, 3437, 1595, 1561 cm^{-1}. **^{1}H NMR** ($CDCl_3$): 7.51 (d, *J*=8.0 Hz, 1H, H-3'), 7.20-7.18 (m, 2H, H-5', H-6'), 7.04 (ddd, *J*=8.0, 4.6, 4.6 Hz, 1H, H-4'), 6.59 (s, 1H, H-8), 6.52 (s, 1H, H-5), 4.21 (dd, *J*=9.6, 4.2 Hz, 1H, H-1), 3.78 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.28 (dd, *J*= 13.6, 4.2 Hz, 1H, $ArCH_2CHNH$), 3.19 (dt, *J*= 12.4, 6.0 Hz, 1H, H-3), 2.96 (dd, *J*= 13.5, 9.6 Hz, 1H, $ArCH_2CHNH$), 2.91 (dd, *J*= 12.3, 5.4 Hz, 1H, H-3), 2.69 (t, *J*=5.9 Hz, 2H, H-4). **^{13}C NMR** ($CDCl_3$): 147.6, 147.1 (C-6, C-7), 138.6 (C-2'), 133.0 (C-3'), 132.0 (C-6'), 130.0 (C-1'), 128.2 (C-4'), 127.4 (C-5'), 126.9 (C-9), 124.9 (C-10), 111.7 (C-5), 109.7 (C-8), 55.8 (OCH_3), 55.8 (OCH_3), 54.8 (C-1), 43.0 ($ArCH_2CHNH$), 39.8 (C-3), 29.1 (C-4). **HRMS** calcd, for $C_{18}H_{21}NO_2Br$: 362.0756, Found 362.0757.

1-(2-Bromobenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline-2-carbaldehyde (8)

A solution of 1-(2-bromobenzyl)-1,2,3,4-tetrahydro-6,7-dimethoxy isoquinoline (7) (300 mg, 0.83 mmol) in ethyl formate (2.78 mL) was heated to 60°C. After 3 h, ethyl formate was removed under reduced pressure to give the crude product. Purification of the crude product by flash column chromatography using ethyl acetate/hexane (2:3) gave 1-(2-bromobenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline-2-carbaldehyde (8) (0.32 g, 85%) as a white solid. Recrystallization of compound **8** from ethanol gave a white crystal with m.p. 133 to 134°C. **FTIR** (KBr), υ_{max}, 1654, 1520 cm^{-1}. **^{1}H NMR** ($CDCl_3$): **Major isomer (*E* isomer)**: 7.53 (dd, *J*=7.6, 1.2 Hz, 1H, H-3'), 7.50 (s, 1H, CHO), 7.18 (ddd, *J*=7.6, 7.5, 1.2 Hz, 1H, H-5'), 7.07 (ddd, *J*=7.6, 7.6, 1.7 Hz, 1H, H-4'), 6.96 (dd, *J*=7.5, 1.7 Hz, 1H, H-6'), 6.65 (s, 1H, H-8), 6.56 (s, 1H, H-5), 4.71 (dd, *J*=10.2, 4.0 Hz, 1H, H-1), 4.43 (ddd, *J*=13.2, 6.2, 2.3 Hz, 1H, H-3), 3.80 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.27 (dd, *J*=13.9, 4.0 Hz, 1H, $ArCH_2CHNH$), 3.17 (ddd, *J*=13.2, 11.4, 4.8 Hz, 1H, H-3), 3.02 (dd, *J*=13.9, 10.2 Hz, 1H, $ArCH_2CHNH$), 2.81 (dd, *J*=11.4, 6.2 Hz, 1H, H-4), 2.70 (dd, *J*=4.8, 2.3 Hz, 1H, H-4). **Minor**

isomer (*Z* isomer): 7.98 (s, 1H, CHO), 7.74 (dd, *J*=8.0, 1.1 Hz, 1H, H-3'), 7.17 (ddd, *J*=7.6, 6.8, 1.1 Hz, 1H, H-5'), 7.11 (dd, *J*=7.6, 2.2 Hz, 1H, H-6'), 7.01 (ddd, *J*=8.0, 6.8, 2.2 Hz, 1H, H-4'), 6.49 (s, 1H, H-8), 6.34 (s, 1H, H-5), 5.59 (dd, *J*=8.4, 6.0 Hz, 1H, H-1), 3.77 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 3.62-3.58 (m, 2H, H-3), 3.26 (dd, *J*=13.7, 6.0 Hz, 1H, ArC$\underline{H}_2$CHNH), 3.08 (dd, *J*=13.7, 8.5 Hz, 1H, ArC$\underline{H}_2$CHNH), 2.85 (dd, *J*=11.3, 6.2 Hz, 1H, H-4), 2.65 (dd, *J*=4.7, 2.3 Hz, 1H, H-4). **^{13}C NMR** ($CDCl_3$): **Major isomer (*E* isomer)**: 161.2 (CHO), 148.3, 147.6 (C-6, C-7), 136.6 (C-2'), 133.0 (C-3'), 132.1 (C-6'), 128.9 (C-4'), 127.8 (C-5'), 127.3 (C-1'), 125.9 (C-9), 124.4 (C-10), 111.5 (C-5), 109.7 (C-8), 56.5 (C-1), 55.9 (OCH_3), 55.8 (OCH_3), 43.4 (Ar$\underline{C}H_2$CHNH), 34.3 (C-3), 27.6 (C-4). **Minor isomer (*Z* isomer)**: 161.2 (CHO), 148.0, 147.4 (C-6, C-7), 137.1 (C-2'), 132.7 (C-3'), 131.6 (C-6'), 128.3 (C-4'), 127.2 (C-5'), 127.0 (C-1'), 125.3 (C-9), 124.9 (C-10), 111.2 (C-8), 110.2 (C-5), 55.8 (OCH_3), 55.7 (OCH_3), 50.8 (C-1), 41.6 (Ar$\underline{C}H_2$CHNH), 40.3 (C-3), 29.1 (C-4). **HRMS** calcd, for $C_{19}H_{21}NO_3Br$: 390.0705, Found 390.0705.

(±)-*N-Formylnornuciferine* (1a)

A mixture of 1-(2-bromobenzyl)-3,4-dihydro-6,7-dimethoxyisoquinoline-2-carbaldehyde (8) (50 mg, 0.13 mmol), tricyclohexylphosphine (36 mg, 0.13 mmol), sodium acetate (33 mg, 0.41 mmol) and palladium acetate (14 mg, 0.06 mmol) in dimethylacetamide (1 mL) under nitrogen atmosphere was heated at 110°C for 24 h. The reaction mixture was filtered through a celite pad and the solvent was evaporated under reduced pressure. The resulting residue was purified by flash column chromatography using ethyl acetate/hexane (2:3) and then was recrystallized from methanol to give *N*-formylnornuciferine (1) (28 mg, 78%) as a colorless crystal, m.p. 222 to 224°C. **FTIR** (KBr), u_{max}, 1668, 1587 cm^{-1}. **^{1}H NMR** ($CDCl_3$): **Major isomer (*Z* isomer)**: 8.35 (d, *J*=8.0 Hz, 1H, H-11), 8.19 (s, 1H, CHO), 7.31-7.15 (m, 3H, H-10, H-9, H-8), 6.59 (s, 1H, H-3), 4.86 (dd, *J*=13.9, 4.2 Hz, 1H, H-6a), 3.83 (s, 3H, OCH_3), 3.75 (ddd, *J*=12.7, 4.6, 1.8 Hz, 1H, H-5), 3.60 (s, 3H, OCH_3), 3.35 (ddd, *J*=12.7, 12.4, 2.8 Hz, 1H, H-5), 3.05 (dd, *J*=13.7, 4.2 Hz, 1H, H-7), 2.89-2.66 (m, 3H, H-4, H-4, H-7). **Minor isomer (*E* isomer)**: 8.37 (d, *J*=8.0 Hz, 1H, H-11), 8.32 (s, 1H, CHO), 7.31-7.15 (m, 3H, H-10, H-9, H-8), 6.62 (s, 1H, H-3), 4.43 (dd, *J*=14.2, 4.0 Hz, 1H, H-6a), 4.35 (ddd, *J*=12.7, 4.6, 3.6 Hz, 1H, H-5), 3.84 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 3.14 (ddd, *J*=12.7, 10.4, 3.7 Hz, 1H, H-5), 3.12-3.03 (m, 1H, H-7), 2.89-2.66 (m, 3H, H-4, H-4, H-7). **^{13}C NMR** ($CDCl_3$): **Major isomer (*Z* isomer)**: 162.1 (CHO), 152.4 (C-2), 146.0 (C-1), 136.1 (C-7a), 131.5 (C-11a), 128.6 (C-11c), 128.6 (C-8), 128.4 (C-11), 127.9 (C-11b), 127.8 (C-9), 127.1 (C-10), 125.3 (C-3a), 111.5 (C-3), 60.0 (OCH_3), 56.0 (OCH_3), 49.4 (C-6a), 42.0 (C-5), 34.1 (C-7), 31.0 (C-4). **Minor isomer (*Z* isomer)**: 161.9 (CHO), 152.6 (C-2), 145.8 (C-1), 135.4 (C-7a), 131.6 (C-11a), 129.5 (C-11c), 128.7 (C-11), 128.2 (C-8), 127.9 (C-9), 127.5 (C-10), 127.4 (C-11b), 124.8 (C-3a), 111.8 (C-3), 60.1 (OCH_3), 56.0 (OCH_3), 53.5 (C-6a), 37.9 (C-7), 36.1 (C-5), 29.6 (C-4). **MS** (EI), *m/z* (relative intensity): 309 (59), 252 (20), 264 (22), 251 (100).

***In vitro* cardiotonic testing**

Male Wistar rats weighing 250-300 g were sacrificed by a sharp blow on the head and exsanguination. In each rat, the heart was quickly excised, placed and swirled for a few seconds in a beaker containing Krebs-Henselet solution (Composition (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0; glucose 5.8, pH 7.4) and gassed with carbogen (95% O_2 and 5% CO_2), then transferred to a Petri dish containing Krebs-Henselet solution. The atria were removed and cut into right and left sides. They were then mounted in an organ-bath containing 20 mL of Krebs-Henselet solution

gassed with carbogen and kept at 37°C. The right atria, which were beating spontaneously, were allowed to equilibrate until a stable rate was observed. The left atria were electrically stimulated with a square wave pulse (5 V strength and 5 ms duration) to beat at a constant rate of 250 per min. The force of contraction and rate were recorded with an isometric force transducer (Mac Lab) connected to a Mac Lab Bridge Amplifier and the results were recorded or printed by a Macintosh LC 457 microcomputer. The test compounds were dissolved in dimethylsulfoxide (1 mg/ 0.5 mL). This solution (10-20 μL) was introduced to the organ bath. After 30 min, the force of contraction and heart rate were recorded for 30 min.

RESULTS AND DISCUSSION

Chemistry

To synthesize (±)-*N*-formylnornuciferine (1a), a palladium-catalyzed coupling reaction was used as a key step (Figure 3). The coupling reaction between acid chloride (3), derived from 2-bromophenylacetic acid (2), and homoveratrylamine (4) produced a high yield of amide (5). Bischler–Napieralski cyclization of **5** gave dihydroisoquinoline (6), which was not stable in air. Therefore, it was reduced using sodium borohydride to give **7** as a racemic mixture without prior purification. Formylation of **7** by refluxing in ethyl formate afforded **8** with a yield of 81%. A palladium-catalyzed coupling reaction of **8** with

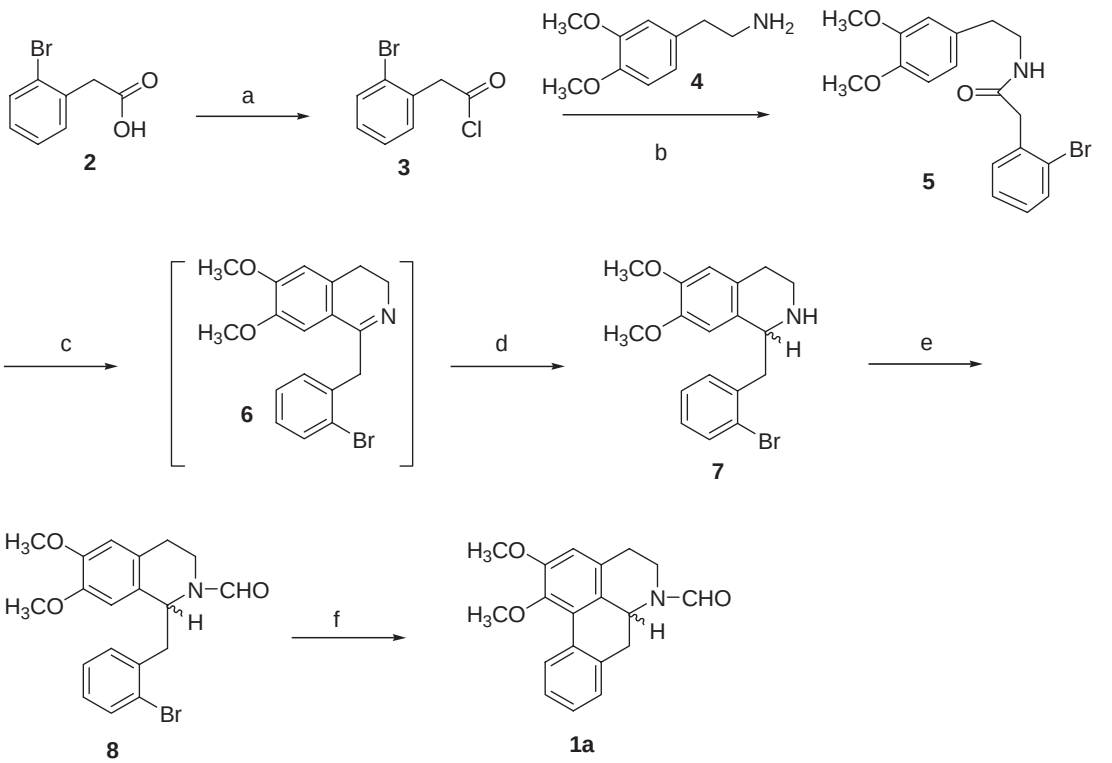

Figure 3 Synthesis of (±)-*N*-formylnornuciferine (1a)
Reagents and conditions: a) $(COCl)_2$, DMF (cat.), benzene, rt, 2 h; b) Homoveratrylamine (4), Na_2CO_3, 1:1 v/v CH_2Cl_2: H_2O, rt, 3h, (92%, two steps); c) $POCl_3$, CH_2Cl_2, reflux, 4 h; d) $NaBH_4$, MeOH, rt, 2 h, (81%, two steps); e) HCOOEt, reflux, 3 h, (81%); f) $Pd(OAc)_2$, PCy_3, NaOAc, DMA, 110°C, 14 h, (78%).

palladium acetate and tricyclohexylphosphine, sodium acetate in dimethyl acetamide at 110°C gave *N*-formylnornuciferine (1a) as a target molecule in a racemic form.

Cardiotonic activity

The racemic mixture of (±)-*N*-formylnornuciferine (1a) was tested for cardiotonic activity on an isolated rat heart. The results showed that its activity was not the same as for the natural aporphine alkoloid isolated from *Tinospora crispa* (Sunthikawinsakul, 2005). (-)-*N-Formylnornuciferine* (1), isolated from *T. crispa*, exhibited a significant increase in the force of contraction on the atrium of an isolated rat heart with no change in the heart rate (Sunthikawinsakul, 2005; Kongkathip *et al*., 2007), whereas the racemic mixture of (±)-*N*-formylnornuciferine (1a), obtained from synthesis in the current study, showed a decrease in the force of atrium contraction, with a small decrease in the heart rate (Figure 4).

The difference in the activities might have resulted from the fact that the synthetic compound contained a racemic mixture of (±)-*N*-formylnornuciferine (1a), while the alkaloid isolated from *T. crispa* was a single enantiomer of (-)-*N*-formylnornuciferine (1) (Figure 5). The synthesis of (-)-*N*-formylnornuciferine (1) with *R* configuration of C-6a is in progress.

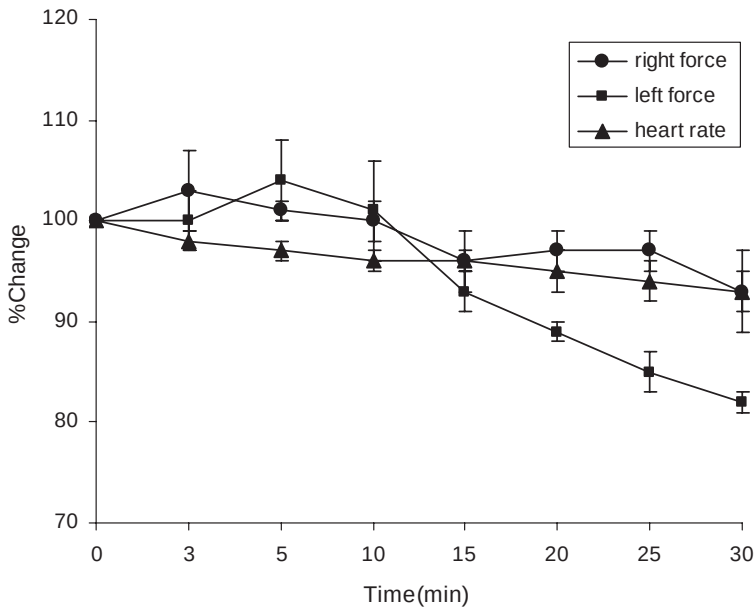

Figure 4 Cardiotonic effects of (±)-*N*-formylnornuciferine (1a) on an isolated rat heart.

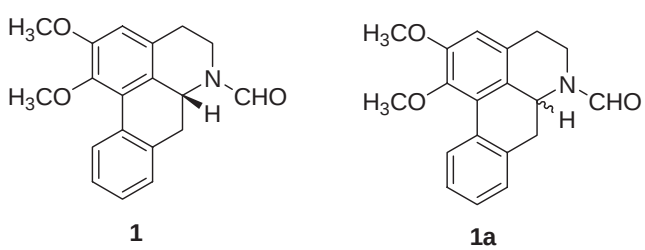

Figure 5 Structures of (-)-*N*-formylnornuciferine (1) and (±)-*N*-formylnornuciferine (1a).

CONCLUSION

The synthesis of (±)-*N*-formylnornuciferine (1a) was accomplished with a palladium-catalyzed coupling reaction as a key step. (±)-*N*-Formylnornuciferine (1a) was obtained in six steps from a commercially available homoveratrylamine with an overall yield of 50%. (±)-*N*-Formylnornuciferine (1a) in racemic form was evaluated for cardiotonic activity. The results showed that the synthetic alkaloid in racemic form exhibited different activity from the natural alkaloid. The synthetic alkaloid in racemic form exhibited a decrease in the heart rate, while the natural alkaloid exhibited a significant increase in the force of contraction with no change in the rate.

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