Synthesis and Insecticidal Activity of Rotenone Analogues

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Summary: Rotenone, one of traditional botanical insecticide, has been used more than one hundred years. A variety of rotenone derivatives were designed and synthesized in recent years due to environmental benign character and not easy to generate insecticide resistance. This paper described the molecular design, synthesis, and insecticidal activities of a series of rotenone analogues and 2-substituted rotenone derivatives. The preliminary bioassay showed that isorotenone and 2-rotenone nicotinate is equal to rotenone's against *Musca domestica*.

Keywords: Rotenone analogues, Organic synthesis, Insecticidal activity.

Introduction

Rotenone, as an isoflavonoid of plant secondary metabolity, is the most important and effective insecticidal constituent of derris root, which was first isolated in 1895 [1], and it is a specific inhibitor of mitochondrial complex I by blocking electron transfer in nicotinamide adenine dinucleotide diaphorase-quinone (NADH-Q) reductase [2, 3]. Rotenone had been used for hundreds of years in Asia and South America to stupefy fish in rivers, and also had been used to control aphids, thrips, suckers, and other insects on fruits and vegetables by farmer since one hundred years ago. However, it showed a short persistence because it can be easily decomposed in the presence of light [4]. The class of rotenoids has received renewed attentions due to their anticancer properties, inherent environmental benign character and low insecticide resistance. Although rotenone is a respiratory enzyme inhibitor, and acting between NAD^+ and coenzyme Q, resulting in failure of the respiratory functions [5], the relationship between structure and activity of rotenone and respiratory enzyme is still not clear.

Rotenone belongs to a class of compounds called the rotenoids which all possess the cis-fused tetrahydrochromeno[3,4-b]chromene nucleus and three stereogenic centres(Fig. 1), which presents a challenging synthetic target. Many organic chemists have been trying to synthetize the target molecule by non-stereoselective and stereoselective methods, recently the stereoselective synthesis of rotenone was be completed [6]. Hence, it laid a foundation for the relationship between structure and activity of rotenone and respiratory enzyme.

In our previous work [7, 8], we synthesized rotenone-O-monosaccharide derivatives, rotenone α-oxime, rotenone-hydrazone and further assessed their phloem transport properties and biology activities. In this study, we designed and synthesized a serial of rotenone analogues and 2-substituted rotenone derivatives with different substituent groups. The contact toxicity of these derivatives toward *Musca domestica* by topical application and *Aedes albopictus* by immersion method was investigated. These results could offer some implications on analysis the relationship between structure and activity as pesticides and obtain insecticidal rotenone analogs with important values on application.

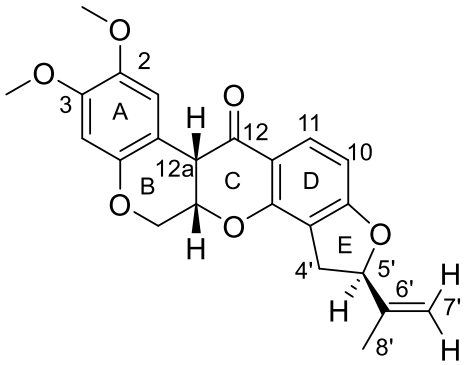

Fig. 1: The structure of rotenone.

Experimental

Instruments

All reagents and solvents were purchased from commercial sources and used without

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purification. Chromatography was made on silica gel purchased from Qingdao Ocean Chemical Co., Ltd. (silica gel 60, 0.063-0.200 mm). Analytical thin-layer chromatography (TLC) was carried out using 0.2 mm commercial silica gel plates (silica gel 60 with fluorescent indicator UV_{254}). Mass spectra were recorded on an Esquire 3000 Plus equipment, in both positive or negative Electron Spray ionization (ESI) mode and are reported as *m/z*. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded with a Bruker Advance instrument at 500 or 600 MHz. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from used solvents, specified for each product. Coupling constants are reported in Hertz (Hz).

*Synthesis of 2-De-O-methylrotenone (**A**) and 2, 3-de-O-dimethylrotenone (**B**)[9]*

Rotenone (3.0 g) in dry dichloromethane (15 mL) was added to boron tribromide (1.45 mL) in dichloromethane (15 mL), kept below -5 ℃. After 5 min the solution was evaporated, the residue cooled in ice was treated in sequence with methanol (30 mL), acetone (90 mL), and saturated aqueous sodium hydrogen carbonate (120 mL). The mixture was stirred at room temperature for 2h, then diluted with distilled water, neutralized (10% hydrochloric acid), and extracted with chloroform. The extracts were washed, dried, and evaporated. The residue was purified by P.L.C (2-De-O-methylrotenone (**A**), 1.55 g, 31%; 2, 3-de-O-dimethylrotenone (**B**), 0.17 g, 17%) (Fig. 2).

Compound A ((2R,6aS,12aS)-8-Hydroxy-9-methoxy-2-(prop-1-en-2-yl)-1,2,12,12a-tetrahydrochromeno [3,4-b]furo[2,3-h]chromen-6(6aH)-one): ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.80 (1H, d, *J*=10.7Hz, ph-H); 6.80(1H, s, ph-H); 6.47(1H, d, *J*=10.7Hz, ph-H); 6.41(1H, s, ph-H); 5.20(1H, t, *J* =11.3Hz, H-20); 5.15(1H, s, OH); 5.04(1H, s); 4.90(2H, s, CH_2); 4.57(1H, d, *J* =15.1Hz, 3.8 Hz, CH_2); 4.15(1H, d, *J* =15.1Hz, CH_2); 3.80(1H, s); 3.79(3H, s, CH_3); 3.29(1H, dd, *J* =19.7Hz, 12.3Hz, CH_2); 2.92(1H, dd, *J* =19.7 Hz, 10.2 Hz, CH_2); 1.74(3H, s, CH_3). ESIMS: m/z 381[M + H]$^+$, 419 [M + K]$^+$.

Compound B ((2R,6aS,12aS)-8,9-Dihydroxy-2-(prop-1-en-2-yl)-1,2,12,12a-tetrahydrochromeno[3,4-b]furo[2,3-h]chromen-6(6aH)-one)**:** ^{1}H NMR(500 MHz, $CDCl_3$): δ_H 7.76 (1H, d *J* =11.0Hz, ph-H); 6.84 (1H, s, ph-H); 6.47 (1H, s, ph-H); 6.44 (1H, s, ph-H); 5.23 (1H, t, *J* =11.0Hz); 5.05 (1H, s); 4.88 (2H, t, *J* =4.0Hz, CH_2); 4.57 (1H, dd, *J* =15.5Hz, 4.0Hz, H-9, CH_2); 4.14 (1H, d, *J* =15.0Hz, CH_2); 3.80 (1H, d, *J* =5.0Hz); 3.30 (1H, dd, *J* =19.5Hz, 12.5Hz, CH_2); 2.92 (1H, dd, *J* =19.5Hz, 10.5Hz, CH_2); 1.74 (3H, s, CH_3)。ESIMS: m/z 367[M + H]$^+$, 389 [M + Na]$^+$, 405 [M + K]$^+$.

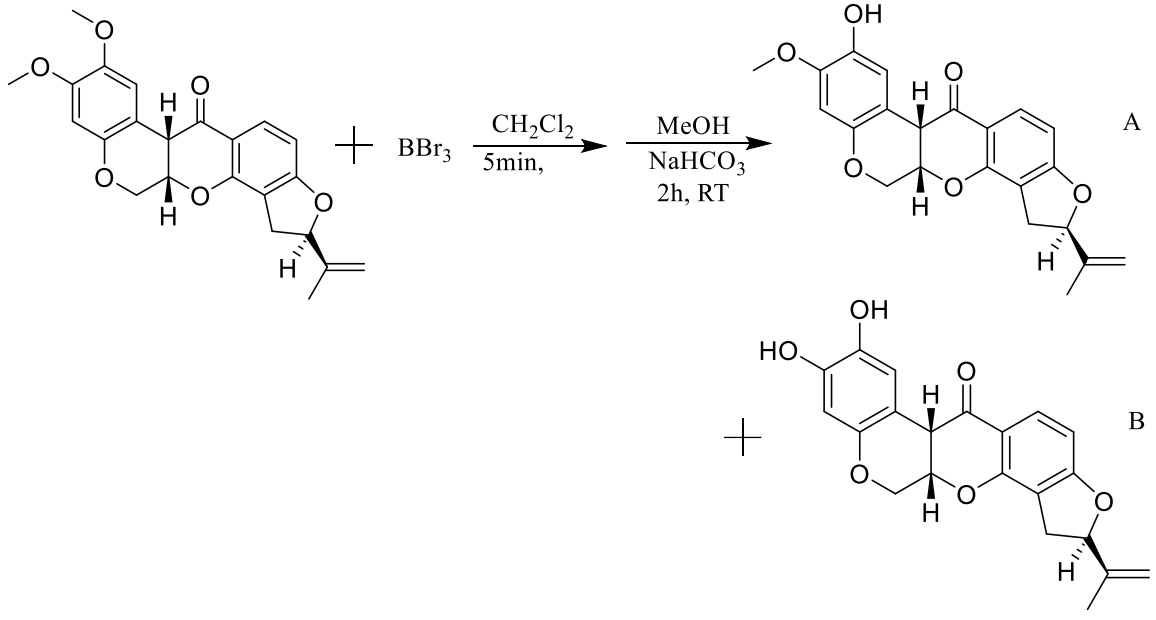

Fig. 2: The synthesis scheme of compound **A** and **B.**

*Synthesis of 12-hydroxyrotenone (**C**)*

Rotenone (1.0 g), 96% sodium borohydride (0.33 g) in 20 mL methanol kept 50 ℃ were refluxed for 2h, water was added until crystallization commenced. The resulting 12-hydroxy- rotenone was crystallized from methanol (0.98 g, 98%), m.p. 98-100 ºC (Fig. 3).

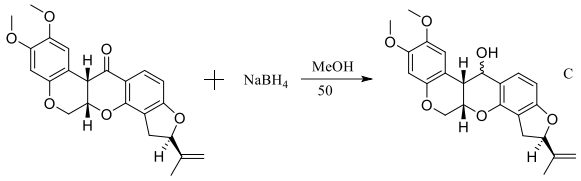

Fig. 3: The synthesis scheme of compound **C.**

Compound C ((2R,6aR,12aS)-8,9Dimethoxy-2(prop-1-en-2-yl)-1,2,6,6a,12,12a- hexahydrochromeno [3,4-b]furo[2,3-h] chromen-6-ol): ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 7.02 (1H, d, *J* =8.4Hz, ph-H); 6.67 (1H, s, ph-H); 6.52 (1H, s, ph-H); 6.44 (1H, s, ph-H); 5.34 (1H, t, *J* =9.0Hz); 5.16 (1H, s); 5.06 (2H, s, CH_2); 4.70 (1H, t, *J* =9.0Hz, CH_2); 4.48 (1H, t, *J* =7.8Hz); 4.31 (1H, d, *J* =13.2Hz, CH_2); 3.78 (3H, s, CH_3); 3.76 (3H, s, CH_3); 3.51 (1H, s); 3.18 (1H, d, *J* =9.6Hz, CH_2); 2.80 (1H, d, *J* =18.0Hz, CH_2); 1.89 (3H, s, CH_3). ^{13}C NMR(150 MHz, $CDCl_3$); δ_C 161.8; 149.6; 149.3; 149.2; 143.8; 143.8; 130.4; 113.7; 112.8; 112.0; 111.2; 108.7; 102.7; 100.7; 86.6; 69.1; 66.3; 65.0; 56.5; 55.9; 38.1; 31.9; 17.2.

*Synthesis of rotenone α-oxime(**D**)[8, 10]*

Rotenone (5.0 g), hydroxylamine hydrochloride (5.0 g) and sodium acetate (6.0 g) in ethyl alcohol (200 mL) were refluxed for 10 h, and then water was added until crystallization commenced. The resulting rotenone α-oxime ((2R,6aR,12aS)-8,9-dimethoxy-2-(prop-1-en-2-yl)-1, 2,12,12a-tetrahydrochromeno[3,4-b]furo[2,3-h]chro men-6(6aH)-one oxime) was crystallized from alcohol (4.55 g, 91%), m.p. 247-249 ºC (Fig. 4).

*Synthesis of rotenone-hydrazone(**E**)*

Rotenone (1.0 g), sodium acetate(1.2 g) and 80％hydrazine hydrate(2.5 mL) in ethyl alcohol (20 mL) keep 80 ºC, were refluxed for 5 h, and then water was added until crystallization commenced. The resulting rotenone-hydrazone was crystallized from alcohol (0.91 g, 91%), m.p. 232-234℃ (Fig. 5).

Compound E (((2R,6aR,12aS)-8,9-dimethoxy-2-(prop-1-en-2-yl)-1 ,2,12,12a-tetrahydrochromeno [3,4-b]furo[2,3-h]chromen-6(6aH)-ylidene)hydrazine): ^{1}H NMR (400 MHz,$CDCl_3$): δ_H 7.44 (1H, d, *J* =8.0Hz, ph-H); 6.73 (1H, s, ph-H); 6.48 (1H, d, *J* =8.0Hz, ph-H); 6.41 (1H, s, ph-H); 5.24 (1H, t, *J* =8.4Hz); 5.07 (2H, s, CH_2); 4.40 (1H, d, *J* =12.0Hz, CH_2); 4.19 (1H, d, *J* =12.0Hz, CH_2); 3.76 (3H, s, CH_3); 3.52 (3H, s, CH_3); 3.15 (1H, d, *J* =9.6Hz, CH_2); 2.86 (1H, d, *J* =18.0Hz, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ_C162.3; 158.5; 155.6; 148.9; 148.7; 143.7; 129.0; 113.8; 113.3; 112.1; 111.8; 109.0; 101.6; 100.5; 89.9; 87.1; 56.0; 55.8; 42.8; 32.7; 31.6; 29.8; 18.0.

*Synthesis of isorotenone (**F**) and 6'-hydroxy-6',7'-dihydrorotenone (**G**)*

0.3 g carbamide, 10 mL acetic acid and 5.0 mL 98% sulfuric acid were fixed in three mouths flask. After stirred for 30 min at 50ºC, then 1.24 g rotenone was added, and the organic material was extracted with dichloromethane. After drying and removed of the solvent, isorotenone (0.42 g, m.p. 180 ºC) and 6'- hydroxy- 6',7' - dihydrorotenone (0.61 g, m.p. 185 ºC) were obtained which were purified by P.L.C (Fig. 6).

Compound F ((6aS,12aS)-2-isopropyl-8,9-dimethoxy-12,12a-dihydrochromeno [3,4-b]furo[2,3-h]chromen-6(6aH)-one): ^{1}H NMR (500 MHz,$CDCl_3$): δ_H 7.83 (1H, d, *J* =12.0Hz, ph-H); 7.07 (1H, d, *J* =6.0Hz, ph-H); 6.76 (1H, s, ph-H); 6.53 (1H, s, ph-H); 6.46 (1H, s, ph-H); 5.05 (1H, s); 4.70 (1H, dd, *J* =12.0Hz, 3.0Hz, CH_2); 4.24 (1H, d, *J* =12.0Hz, CH_2); 3.92 (1H, d, *J* =6.0Hz); 3.79 (3H, s, CH_3); 3.76 (3H, s, CH_3); 3.05 (1H, dd, *J* =6.0Hz, 3.0Hz); 1.31 (6H, t, *J* =6.0Hz, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$); δ_C 190.0; 165.2; 159.9; 155.2; 149.5; 147.4; 143.8; 122.9; 118.2; 113.3; 110.3; 106.2; 104.6; 100.9; 97.9; 72.7; 66.2; 56.3; 55.6; 44.7; 28.1; 20.81.

Compound G ((6aS,12aS)-2-(2-hydroxypropan-2-yl) -8,9-dimethoxy-1,2,12,12a-tetrahydrochromeno [3,4-b]furo[2,3-h] chromen-6(6aH)-one): ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.80 (1H, d, *J* =10.5Hz, ph-H); 6.74 (1H, s, ph-H); 6.47 (1H, s, ph-H); 6.43 (1H, s, ph-H); 4.91 (1H, t, *J* =4.0Hz); 4.61 (2H, d, *J* =4.0Hz, CH_2); 4.16 (1H, d, *J* =15.0Hz, CH_2); 3.82 (1H, d, *J* =9.5Hz, CH_2); 3.78 (1H, s); 3.74 (6H, s, CH_3); 3.10 (2H, dd, *J* =11.5Hz, 6.0Hz, CH_2); 1.33 (3H, s, CH_3); 1.20 (3H, s, CH_3). ESIMS: m/z 413$[M + H]^+$,435 $[M + Na]^+$, 451$[M + K]^+$。 ESIMS: m/z 411$[M-H]^+$.

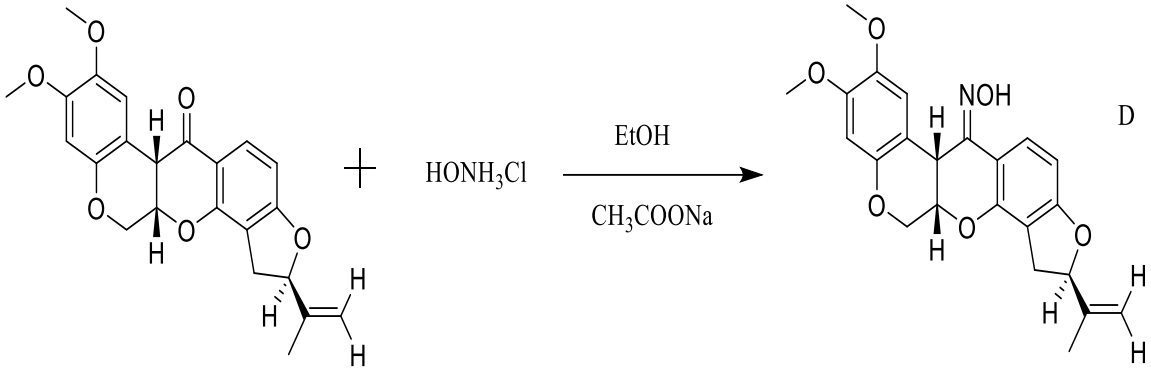

Fig. 4: The synthesis scheme of compound **D.**

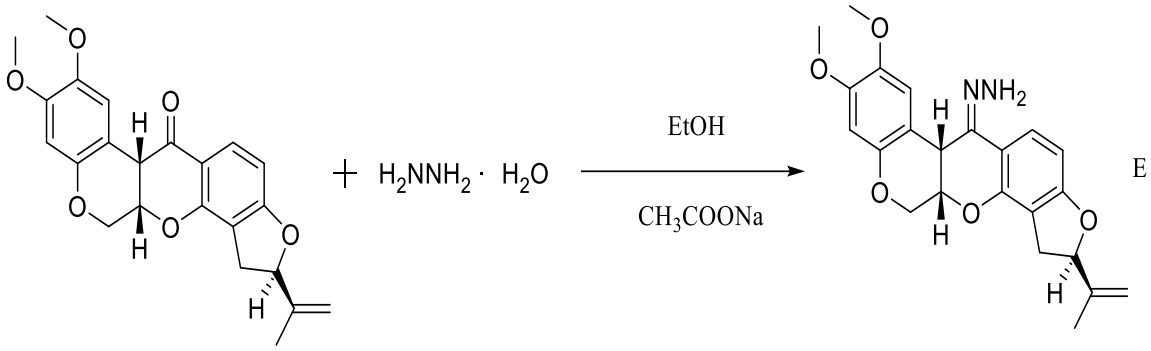

Fig. 5: The synthesis scheme of compound **E.**

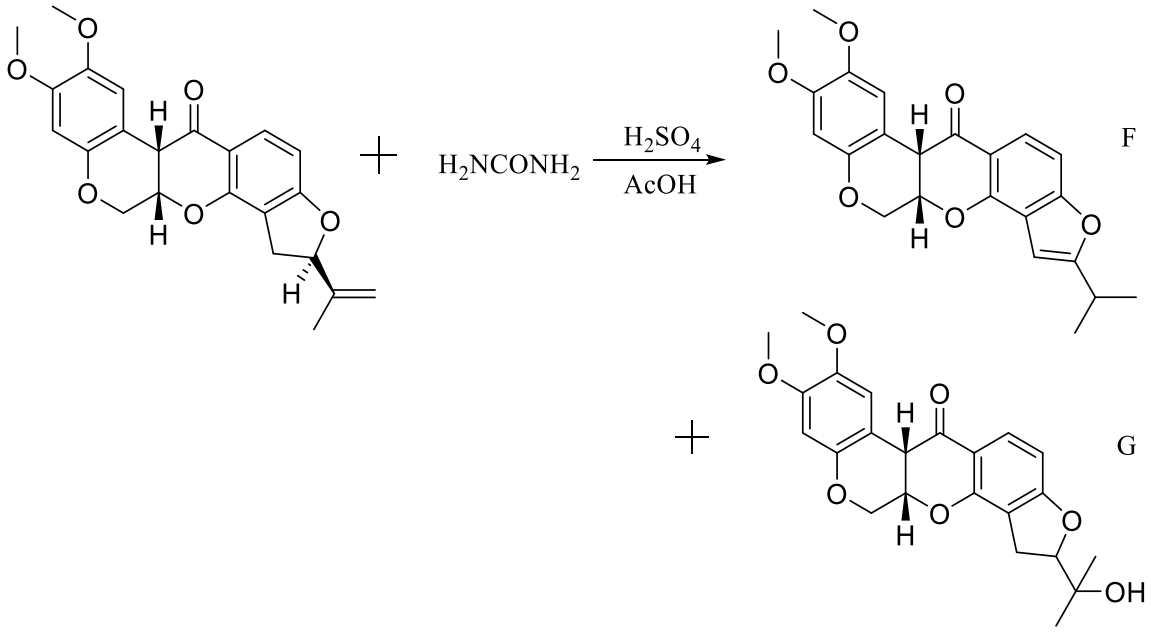

Fig. 6: The synthesis scheme of compound **F** and **G.**

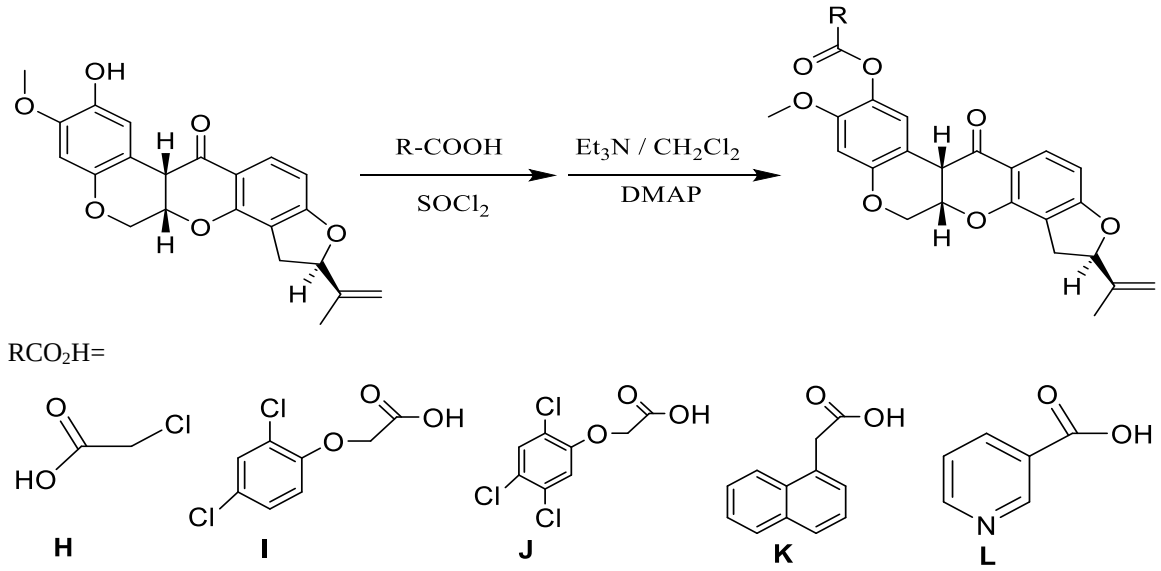

Fig. 7: The synthesis scheme of compound **H-L.**

Synthesis of the compounds **H-L**

The acyl chloride compounds reaction

One mol compounds (monochloroacetic acid, 2,4-dichlorophenocyacetic acid, 2,4,5-trichlorophocyacetic acid, nicotine acid, monochloracetic acid and alpha-naphthyl acetic acid) was placed in a 50 mL round-bottomed flask equipped with a Teflon-coated magnetic stirring bar and a reflux condenser. Total of 1.5 mol thionyl chloride was added in one portion and then the suspension was continuing stirred at reflux for 3 h. At last, the reaction mixture was cooled at room temperature before removing excess thionyl chloride under reduced pressure. The acyl chloride compounds can be obtained.

The esterification reaction

2-De-O-methylrotenone and dichloromethane were added to a 50 mL, three-necked, round-bottomed flask equipped with a Teflon-coated magnetic stirring bar, a thermometer, and a 25 mL, pressure-equalizing dropping funnel connected to a nitrogen flow line. The solution was cooled to 0°C (ice-methanol bath) and treated with mild triethylamine. The dropping funnel was filled with a solution of acyl chloride in dichloromethane (75 mL). This solution was added dropwise in 5 min. The reaction mixture was stirred for 1 hr at room temperature, then transferred to a 100 mL separatory funnel before sequentially washing with water (2 × 40 mL). The mixture was stirred for 20 min and then transferred into a 1000 mL separatory funnel. The organic phase was separated and the aqueous layer was extracted with dichloromethane (2 × 100 mL). The combined organic layers were collected in a 100 mL Erlenmeyer flask and dried over magnesium sulfate under nitrogen. At last, total of esterification products was obtained (Fig. 7).

Compound H ((2R,6aS,12aS)-9-methoxy-6-oxo-2-(prop-1-en-2-yl)-1,2,6,6a,12,12a-hexahydrochromeno[3,4-b]furo[2,3-h]chromen-8-yl 2-chloroacetate): m.p.154-156 ℃ ; ^{1}H-NMR(500 MHz, $CDCl_3$): δ_H 7.80 (1H, d *J* =10.5Hz, ph-H); 6.96 (1H, s, ph-H); 6.48 (2H, t, *J* =6.5Hz, ph-H); 5.23 (1H, t, *J* =11.0Hz); 5.05 (1H, s); 4.91 (2H, s, CH_2); 4.62 (1H, dd, *J* =15.5Hz, 3.5Hz, CH_2); 4.24 (2H, s, CH_2); 4.20 (1H, d, *J* =15.0Hz, CH_2); 3.80 (1H, d, *J* =5.0Hz); 3.73 (3H, s, CH_3); 3.29 (1H, dd, *J* =19.5Hz, 14.5Hz, CH_2); 2.93 (1H, dd, *J* =2.0Hz, 1.0Hz, CH_2); 1.74 (3H, s, CH_3). ESIMS: m/z 457[M + H]$^+$, 479 [M + Na]$^+$, 495 [M + K]$^+$.

Compound I ((2R,6aS,12aS)-9-methoxy-6-oxo-2-(prop-1-en-2-yl)-1,2,6,6a,12,12a-hexahydrochromeno[3,4-b]furo[2,3-h]chromen-8-yl 2-(2,4-dichlorophenoxy)acetate): m.p.155-157 ℃ ; ^{1}H-NMR(500 MHz, $CDCl_3$): δ_H 7.80 (1H, d, *J* =10.5Hz, ph-H); 7.39 (1H, s, ph-H); 7.16 (1H, d, *J* =5.0Hz, ph-H); 6.96 (1H, s, ph-H); 6.87 (1H, d, *J* =11.0Hz, ph-H); 6.81 (1H, d, *J* =11.0Hz, ph-H); 6.50 (1H, d, *J* =11.5Hz, ph-H); 5.22 (1H, t, *J* =11.5Hz); 5.05 (1H, s); 4.90 (2H, s, CH_2); 4.87 (2H, s, CH_2); 4.62 (1H, d, *J* =11.5Hz, CH_2); 4.18 (1H, d, H-9, *J* =15.0Hz, CH_2); 3.80 (1H, d, *J* =4.0Hz); 3.76 (3H, s, CH_3); 3.72 (3H, s, CH_3); 3.29 (1H, dd, *J* =19.5Hz, 12.5Hz, CH_2); 2.93 (1H, dd, *J* =19.5Hz, 10.0Hz, CH_2); 1.74 (3H, s, CH_3 ESIMS: m/z 583 [M + H]$^+$, 605 [M + Na]$^+$, 621 [M + K]$^+$.

Compound J ((2R,6aS,12aS)-9-methoxy-6-oxo-2-(prop-1-en-2-yl)-1,2,6,6a,12,12a-hexahydrochromeno[3,4-b]furo[2,3-h]chromen-8-yl 2-(2,4,5-trichlorophenoxy)acetate): m.p.157-159 ℃; H-NMR(500 MHz, $CDCl_3$): δ_H 7.79 (1H, t, *J* =11.0Hz, ph-H); 7.75 (1H, s, ph-H); 7.45 (1H, s, ph-H); 7.03 (1H, s, ph-H); 6.97 (1H, s, ph-H); 6.49 (2H, t, *J* =5.5Hz, ph-H); 5.23 (1H, t, *J* =11.0Hz); 5.05 (1H, s); 4.91 (2H, s, CH_2); 4.87 (2H, s, CH_2); 4.61 (1H, d, *J* =3.5Hz, CH_2); 4.19 (1H, d, *J* =15.5Hz, CH_2); 3.80 (1H, d, *J* =4.5Hz); 3.74 (3H, s, CH_3); 3.29 (1H, dd, *J* =19.5Hz, 12.5Hz, CH_2); 2.92 (1H, dd, *J* =20.0Hz, 10.0Hz, CH_2); 1.74 (3H, s, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$); δ_C 188.2; 167.4; 166.0; 157.7; 152.6; 152.3; 151.0; 142.9; 133.0; 131.1; 131.0; 130.1; 125.5; 122.6; 121.5; 115.7; 113.2; 113.0; 112.6; 106.0; 105.0; 101.4; 87.8; 71.6; 66.5; 66.1; 56.0; 44.1; 31.2; 17.1.

Compound K ((2R,6aS,12aS)-9-methoxy-6-oxo-2-(prop-1-en-2-yl)-1,2,6,6a,12,12a-hexahydrochromeno[3,4-b]furo[2,3-h]chromen-8-yl 2-(naphthalen-2-yl)acetate): m.p.153-155 ℃; ^{1}H NMR(600 MHz, $CDCl_3$): δ_H 8.06 (1H, d, *J* =8.4Hz, ph-H); 7.82 (1H, d, *J* =5.4Hz, ph-H); 7.78 (1H, d, *J* =5.4Hz, ph-H); 7.76 (1H, d, *J* =5.4Hz, ph-H); 7.52 (1H, d, *J* =7.2Hz, ph-H); 7.48 (1H, d, *J* =1.2Hz, ph-H); 7.46 (1H, d, *J* =1.2Hz, ph-H); 7.41 (1H, d, *J* =8.4Hz, ph-H); 6.92 (1H, s, ph-H); 6.47 (1H, dd, *J* =9.0Hz, ph-H); 6.40 (1H, s, ph-H); 5.18 (1H, t, *J* =9.0Hz); 5.04 (1H, s); 4.90 (1H, s); 4.22 (2H, s, CH_2); 4.11 (2H, d, *J* =7.2Hz, CH_2); 4.07 (1H, d, *J* =6.0Hz, CH_2); 3.72 (1H, d, *J* =3.0Hz, CH_2); 3.53 (3H, s, CH_3); 3.23 (1H, dd, *J* =12.0Hz, 6.0Hz, CH_2); 2.90 (1H, dd, *J* =18.0Hz, 6.0Hz, CH_2); 1.73(3H, s, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$); δ_C 188.3; 169.7; 167.3; 157.7; 151.8; 151.3; 143.0; 134.1; 133.7; 132.1; 130.1; 130.0; 128.6; 128.1; 128.1; 126.1; 125.7; 125.4; 124.0; 121.6; 113.2; 112.9; 112.5; 105.7; 104.9; 101.3; 87.8; 71.7; 66.4; 55.6; 44.2; 38.7; 31.2; 17.1.

Compound L ((2R,6aS,12aS)-9-methoxy-6-oxo-2-(prop-1-en-2-yl)-1,2,6,6a,12,12a-hexahydrochromeno[3,4-b]furo[2,3-h]chromen-8-yl nicotinate): m.p. 108-110 ℃; ^{1}H-NMR(600 MHz, $CDCl_3$): δ_H 9.33 (1H, s, ph-H); 8.82 (1H, s, ph-H); 8.38 (1H, t, *J* =1.8Hz, ph-H); 7.83 (1H, d, *J* =8.4Hz, ph-H); 7.42 (1H, d, *J* =4.8Hz, ph-H); 7.08 (1H, d, *J* =1.0Hz, ph-H); 6.55 (1H, s, ph-H); 6.51 (1H, d, *J* =12.0Hz, ph-H); 5.25 (1H, s); 5.08 (1H, s); 4.94 (2H, s, CH_2); 4.66 (1H, dd, *J* =3.6Hz, 1.0Hz, CH_2); 4.24 (1H, d, *J* =12.0Hz, CH_2); 3.86 (1H, s); 3.76 (3H, s, CH_3); 3.32 (1H, d, *J* =9.6Hz, CH_2); 2.98 (1H, d, *J* =7.8Hz, CH_2); 1.77 (3H, s, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$); δ_C 188.2; 167.4; 165.6; 157.7; 152.2; 151.2; 143.0; 134.2; 133.6; 132.0; 130.1; 123.6; 121.6; 113.3; 112.9; 112.6; 105.9; 105.0; 101.4; 87.8; 71.7; 66.4; 55.9; 44.2; 38.6; 31.3; 17.1.

Bioassay

Bioactivity assay against Musca domestica

Houseflies, *Musca domestica* L., in this study, were obtained from a laboratory colony maintained in Guangdong Center for Disease Control and Prevention, Guangzhou, China and had been maintained continuously since 2000 without exposure to any insecticides and pathogens. Acute topical toxicity was examined with *M. domestica* adults (5 days after eclosion). A micro-electric applicator delivered amounts of 1 μL of each of the oils in acetone to the pronota of anesthetized (CO_2) flies. In primary experiment, we determined the appropriate ranges of the testing concentrations. Certified acetone was used as control treatment. A minimum of four concentrations were replicated at least three times (30 flies per replication) in the final bioassays. The mortality of houseflies was assessed 24 h.

Bioactivity results showed that the activities of compound **F** and **L** against *Musca domestica* after 24 h were 40.20 mg/L and 47.57mg/L, respectively, which were equal to rotenone. While the compounds **A**, **G**, **I**, **J** and **K** showed significant lower activities than rotenone. All of the compound **B**, **C**, **D**, **E** and **H** lost their activities against *Musca domestica* (Table-1).

Bioactivity assay against Aedes albopictus

The tested insects were the 4th-instar larvae of *A. albopictus* (Skuse) which were obtained from a laboratory colony maintained in Guangdong Center for Disease Control and Prevention, Guangzhou, China and had been maintained continuously since 1995 without exposure to any insecticides and pathogens. Each compound was dissolved and serially diluted with acetone. Each serial solution (0.4 mL) was added to a beaker containing 20 mL of dechlorinated water, and then 30 larvae were transferred into the beaker. The average mortality of each treatment (three replications pretreatment) at each concentration was calculated, and the LC_{50} value which was defined as the concentration causing 50% mortality, was also determined. All the experiments were conducted at least two times with three replicates in each case. The mortality of insects was assessed 24 h after the treatment.

Table-1: Bioactivity of the compounds to *Musca domestica* after 24 h.

Compounds	Linear regressive equation (y=a+bx)	Correlation coefficient(r)	LC_{50}(mg/L)	95% confidence interval of LC_{50}
rotenone	y =0.4280+3.0638x	0.9834	31.06	25.16-38.36
A	y=1.9487+1.5457x	0.9855	94.21	62.94-141.02
B	–[1)]	–	–	–
C	–	–	–	–
D	–	–	–	–
E	–	–	–	–
F	y=-4.0342+5.6315x	0.9829	40.20	36.39-44.41
G	y=2.0034+1.3908x	0.9956	142.75	81.65-249.60
H	–	–	–	–
I	y=2.0921+1.5062x	0.9970	85.23	52.90-137.31
J	y=0.7144+2.2445x	0.9981	81.17	52.84-124.70
K	y=1.1145+2.0291x	0.9953	82.20	60.55-111.59
L	y=1.8853+1.8569x	0.9838	47.57	33.48-67.60

(Note:[1)] "–" indicated that compound treated concentration≤500mg/L is no active to *Musca domestica*..

Table-2: Bioactivity of the compounds to the 4th instar larva of *Aedes albopictus* after 24h.

Compounds	Linear regressive equation (y=a+bx)	Correlation coefficient(r)	LC_{50}(mg/L)	95% confidence interval of LC_{50}
rotenone	y =2.8711+3.0029x	0.9842	5.12	4.46-5.87
A	y=2.0746+1.7584x	0.9919	46.09	34.84-60.98
B	–[1)]	–	–	–
C	y=2.1674+2.5494x	0.9807	12.91	11.12-15.00
D	y=1.6859+2.0942x	0.9874	38.24	30.56-47.86
E	–	–	–	–
F	–	–	–	–
G	–	–	–	–
H	–	–	–	–
I	y=1.6551+1.8329x	0.9946	66.82	50.67-88.11
J	y=1.1989+2.1254x	0.9935	61.44	48.51-77.83
K	y=1.7873+2.0949x	0.9658	34.17	28.20-41.41
L	–	–	–	–

Note:[1)] "–" indicated that compound treated concentration≤500mg/L is no active to the 4th instar larva of *Aedes albopictus*

The compounds treated to the 4th instar larva of *Aedes albopictus* after 24h, the LC_{50} values of rotenone was 5.12 mg/L, while the LC_{50} of compound A, C, D, I, J, and K were 46.09 mg/L, 12.91 mg/L, 38.24 mg/L, 66.82 mg/L, 61.44 mg/L and 34.17 mg/L, respectively. All of the compound B, E, F, G, H and L lost their activities against *Aedes albopictus* (Table-2).

Results and Discussion

As a botanical insecticide, rotenone has been used for hundreds of years, but it was mainly used as a fish poison [11]. Rotenone is commonly sold as a dust containing 1% to 5% active ingredients for home and garden use, but liquid formulations used in organic agriculture can contain as much as 8% rotenone and 15% total rotenoids [12]. Rotenone is presently used only on a few of crops due to its high toxicity to fish [13]. Meanwhile, the safety of rotenone has recently been called into question because of inducer of Parkinson's disease by acute exposure and the persistence of rotenone on food crops [12]. However, there remains some interest in exploiting low toxicity insecticide as lead.

The present study explored the efficiency of 12 rotenone analogues. Lethal doses for topical and immersion applications against *Musca domestica* and *Aedes albopictus* were detected, respectively. Rotenone consists of a five-ring structure A- to E-rings, and has three chiral center (6a-C, 12a-C, 5'-C), natural rotenone's stereochemistry is 6aS, 12 aS, 5'R, that be proved by Buchi *et al.*[14], Burgos and Redfearn (1965) have shown that the bent form of rotenone at the face of contact between the B and C rings is very important for inhibiting the NDH activity of mammalian mitochondria, indicating that the bent form is essential for the activity[15]. This conclusion is supported by the observation of many previous researches. Hideki Ueno *et al.* (1996) using purified rotenone stereoisomers (5'α- and 5' β-epirotenones) confirmed this notion [16]. 12-hydroxyrotenone, rotenone a-oxime and rotenone-hydrazone are rotenone derives which have different chemical groups of rotenone in 12-position. Our results indicated that 12-hydroxyrotenone, rotenone a-oxime and rotenone-hydrazone lost their activity against *Musca domestica*, whereas the LC_{50} value of 12-hydroxyrotenone was 12.91 mg/L. which may be caused by compounds have different water-solubility or different manner of inhibition. The result is the same with the apparent inhibiroty potency of the derivative lacking the 12-C=O group in the C-ring is completely retained with bovine complex I[16]. Besides the stereochemical properties of rotenone, Ueno *et al.* (1996) investigated the effects of the substituents in the A-ring on the activity, it was noteworthy that the rotenone derivatives possessing other substitution patterns do not necessarily lose all activity [16]. We use 2-de-O-methylrotenone conjugates some organic

acids on the 2-position, that containing 2, 4-dichlorophenocyacetic acid, 2, 4, 5-trichlorophocyacetic acid, nicotine acid, mono-chloroacetic acid and alpha-naphthyl acetic acid. Mostly of the new compounds showed lower activities than that of rotenone. Whereas 2-rotenone nicotinate has insecticidal activity to *Musca domestica* to a certain extent.

Isorotenone and 6'-hydroxy-6',7'-dihydrorotenone are rotenone derivatives which different from rotenone in E-ring. The bioactivity results showed that isorotenone has equal to rotenone's, and 6'-hydroxy-6',7'-dihydrorotenone has lower than that of rotenone against *Musca domestica*. Whereas, the two compounds losted their activity against *Aedes albopictus*.

It is important to study rotenone by modifying its structure or synthesize rotenone derivatives possessing excellent bioactivities and low toxicity to mammal according to the structure-activity relationship. The results in this paper indicated a definite structure-activity or toxicity relationship of rotenone derivatives which needs to be explored further. Rotenone derivatives of high efficiency and low toxicity to be screened out for further development to use organic agriculture for control pests.

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