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SEMISYNTHESIS AND ANTIFEEDANT ACTIVITY OF NEW ACYLATED DERIVATIVES OF TUTIN, A SESQUITERPENE LACTONE FROM *CORIARIA SINICA*

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Abstract –Three sesquiterpene lactones, tutin (**1**), coriatin (**2**), and corianin (**3**), were isolated from the achenes of *Coriaria sinica* Maxim, and corianin (**3**) being for the first time obtained. Moreover, three new derivatives (**1a-c**) were prepared by acylation at the 2-position of tutin (**1**). Structures of these compounds were identified and characterized by spectroscopic and X-ray crystallographic analysis. Tutin and acylated analogs thereof showed antifeedant activity against *Mythimna separata*, and the most potent was 2-*iso*-butenoyltutin (**1b**).

INTRODUCTION

The shrub *Coriaria sinica* Maxim. (Coriaceae) is a Chinese herbal medicine indigenous to China, which was characterized by sesquiterpene lactones.[1] Of these metabolites coriamyrtin and tutin were found to be major convulsant toxins used to treat schizophrenia.[1] Recently, we demonstrated that a crude extract of the achenes, leaves, and fruits of *C. sinica* displayed marked antifeedant and stomach toxicity effects on several forest pests such as *Stilpnotia candida* and *Arge captive*.[2] In order to search for insecticidal substances from *C. sinica* and to explore their structure-insecticidal activity relationships, we embarked upon an investigation into toxic secondary metabolites derived from this species. Three highly oxygenated sesquiterpene lactones with picrotoxane skeleton, i.e. tutin (**1**), coriatin (**2**), and corianin (**3**), were isolated from achenes of this plant, and their structures elucidated by spectral and X-ray crystallographic analysis. In addition, we prepared three new derivatives (**1a-c**) with different acylated

substituents at the 2-position of tutin (**1**). In this paper, we herein describe semisynthesis and antifeedant activity of new tutin derivatives, as well as structures of compounds (**1-3**).

RESULTS AND DISCUSSION

The chloroform fraction of the ethanolic extracts of achenes separated from *C. sinica* berries were repeatedly subjected to column chromatography over silica gel, followed by reversed-phase HPLC to give tutin (**1**), coriatin (**2**) and corianin (**3**) (Figure 1). The IR spectra showed an absorption band assignable to the lactone at 1760 or 1774 cm^{-1}. The structure for each compound was most elucidated by a combination of NMR, IR, and MS spectroscopy, as well as by comparison with data reported in literature.[1b,1c,1d,3-5] The exact structures of tutin (**1**) and coriatin (**2**) were further confirmed by the X-ray crystallographic analysis, as shown in Figure 2.

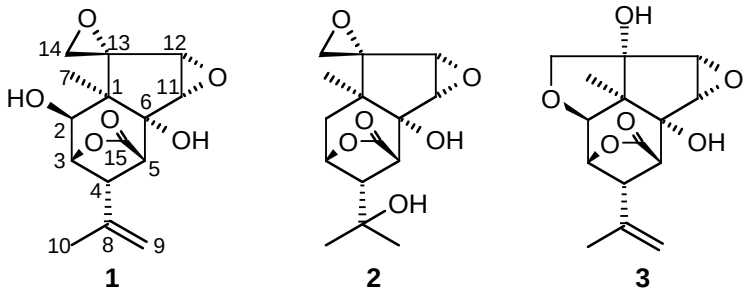


Figure 1. Structures of tutin (**1**), coriatin (**2**) and corianin (**3**)

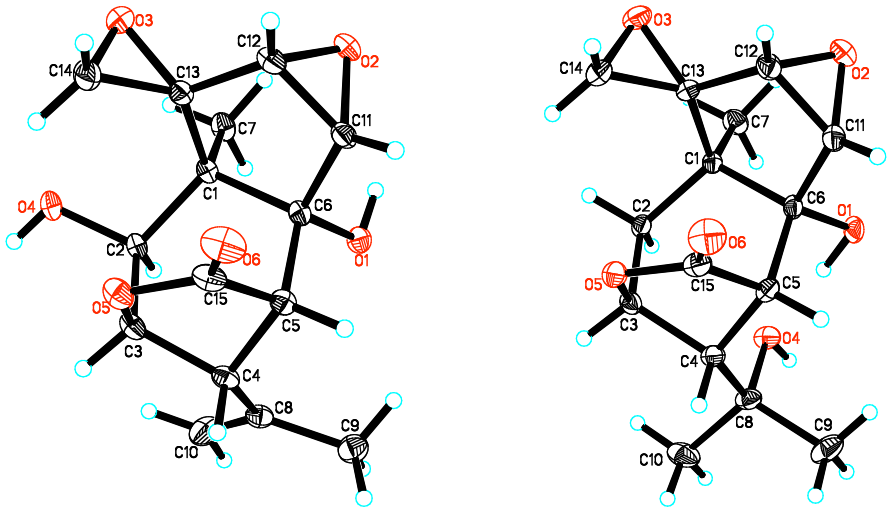
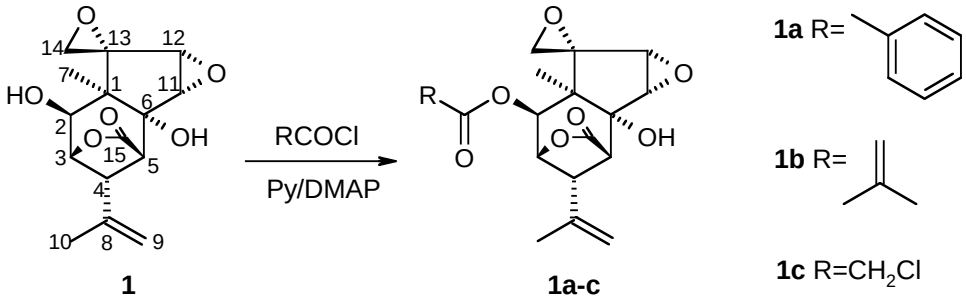


Figure 2. Crystal structures of tutin **1** (left) and coriatin **2** (right)

Bioassay showed that tutin had antifeedant activity against *Mythimna separate*. Therefore, for further examination of the effects of substituents at the 2-position of this compound on antifeeding property, we

planned the synthesis of three tutin derivatives **1a-c,** 2-benzoyltutin (**1a**), 2-iso-butenoyltutin (**1b**), and 2-chloroacetyltutin (**1c**) were obtained through esterification of hydroxyl at the 2-position of tutin with acyl chloride (Scheme 1). Thus, esterification of tutin with benzoyl chloride, *iso*-butenoyl chloride, and chloroacetyl chloride was performed in the presence of pyridine and DMAP at room temperature, the resulting esters **1a**, **1b**, and **1c** was obtained in quantitative yield. All the semisynthetic derivatives of tutin were elucidated and characterized by spectroscopic data (Table 1 and 2).



Scheme 1. Semisynthetic route to compounds **1a-c**

With the compounds in hand, the 24 h and 48 h antifeedant activity of test compounds was assayed by a conventional leaf disk method against the fourth instar larvae of *Mythimna separate* (Walker). It can be seen from Table 3 that all acylated analogues (**1a-c**) derived from tutin (**1**) showed more antifeedant effect than the parent. The most potent was 2-*iso*-butenoyltutin (**1b**) with an antifeeding rate of 74.90%～84.76%, while 2-chloroacetyltutin (**1c**) had moderate activity with an antifeeding rate of 52.80%～63.61%, and 2-benzoyltutin (**1a**) had weak activity. Interestingly, among compounds, test insects exhibited paralytic and narcotic symptom of poisoning after ingestion of **1b** alone.

Table 1 ^{1}H NMR data for derivatives (1a-c) of tutin (1)

Entry	^{1}H NMR δ ppm
1a	1.39 (s, 3H, H-7), 1.98 (s, 3H, H-10), 2.98 (d, J=4.8 Hz, 1H, H-14), 3.21 (d, J=4.3Hz, 1H, H-5), 3.27 (d, J=3.0Hz, 1H, H-11), 3.30 (s, 1H, H-4), 3.82 (d, J=3.0 Hz, 1H, H-12), 3.91(d, J=4.7Hz, 1H, H-14), 5.09 (s, 1H, H-9), 5.11 (d, J=2.3Hz, 1H, H-3), 5.38 (d, J=1.3Hz, 1H, H-9), 5.51 (s, 1H, H-2), 7.46~7.51 (m, 2H, H-2'), 7.60~7.64 (m, 1H, H-3'), 8.01~8.12 (m, 2H, H-1').
1b	1.29 (s, 3H, H-7), 1.95 (s, 3H, H-10), 1.96 (s, 3H, H-19), 2.88 (d, J=4.9Hz, 1H, H-14), 3.17 (d, J=4.2Hz, 1H, H-12), 3.21 (d, J=3.0Hz,1H, H-5), 3.26 (d, J=5.5Hz, 1H, H-4), 3.75(d, J=4.8Hz, 1H, H-11), 3.78 (d, J=3.1Hz, 1H, H-14), 4.95 (d, J=4.8Hz, 1H, H-3), 5.05 (t, 1H, H-9), 5.27 (d, J=1.8Hz, 1H, H-9), 5.35 (s, 1H, H-2), 5.68 (t, 1H, H-18), 6.16 (s, 1H, H-18).
1c	1.31 (s,3H, H-7), 1.94(s,3H, H-10), 2.51 (s, 1H, H-14), 3.18 (d, J=4.4Hz,1H, H-12), 3.20 (d, J=3.0Hz,1H, H-5), 3.26 (s,1H, H-4), 3.71 (s, 1H, H-11), 3.75 (d, J=4.6Hz, 1H, H-17), 3.78 (d, J=3.1Hz, 1H, H-14), 4.12 (s, 1H, H-17), 4.88 (d, J=4.9Hz, 1H, H-2), 5.04 (d, J=1.4Hz, 1H, H-9), 5.05 (d, J=1.4Hz, 1H, H-3), 5.11 (d, J=1.9Hz, 1H, H-9).

Table 2 ^{13}C NMR data for derivatives (1a-c) of tutin (1)

Entry	^{13}C N MR δ ppm
1a	44.8(C-1), 75.3 (C-2), 80.0 (C-3), 48.7 (C-4), 50.0 (C-5), 77.7 (C-6), 22.8 (C-7), 139.9 (C-8), 113.1 (C-9), 19.9 (C-10), 60.3 (C-11), 59.8 (C-12), 65.1 (C-13), 52.5 (C-14), 174.3 (C-15), 128.5 (C-1'), 129.9 (C-2'), 128.8 (C-3'), 133.7 (C-4')
1b	45.1(C-1), 75.3 (C-2), 80.4 (C-3), 49.2 (C-4), 50.4 (C-5), 78.2 (C-6), 23.2 (C-7), 140.3 (C-8), 113.4 (C-9), 20.2 (C-10), 60.7 (C-11), 60.2 (C-12), 65.3 (C-13), 52.7 (C-14), 174.7 (C-15), 167.8 (C-16), 135.7 (C-17), 128.1 (C-18), 18.5 (C-19)
1c	44.6 (C-1), 76.4 (C-2), 79.7 (C-3), 48.6 (C-4), 49.7 (C-5), 77.8 (C-6), 22.8 (C-7), 139.8 (C-8), 112.8 (C-9), 19.9 (C-10), 60.3 (C-11), 59.8 (C-12), 64.8 (C-13), 52.3 (C-14), 173.9 (C-15), 167.3 (C-16), 40.5 (C-17)

Table 3 Antifeedant effects of tutin (1) and its derivatives (1a-c) against 4th –instar *Mythimna separate*

Entry	**1**	**1a**	**1b**	**1c**
24h-antifeedant rate (%)	28.19	40.69	83.02	63.18
48h-antifeedant rate (%)	26.24	30.26	82.34	56.72

EXPERIMENTAL

General. Melting points were measured on a XT5 micro-melting point apparatus and were uncorrected. NMR spectra were recorded on a Bruker DPX-400 spectrometer with acetone-d_6 as the solvent and TMS as internal standard, J in Hz. Chemical shifts are reported in δ (ppm) values. IR spectra were measured on a Thermo Nicolet IR 200 spectrometer using a KBr pellets. UV spectra were measured on a Pharmaspec UV-1700 spectrophotometer. MS were recorded using a Q-TOF MicroTM spectrometer. X-ray data were obtained on a Nonius CCD Kappa diffractometer using Mo KR radiation λ=0.71073 Å. Column chromatography (CC) was performed on silica gel (200-300 mesh) (Qingdao Marine Chemical, Ltd., China). Thin layer chromatography (TLC) was carried out on silica gel 60 GF_{254} (Qingdao Marine Chemical, Ltd., China) plates, and spots were visualized by spraying with 5%H_2SO_4 in ethanol reagent followed by heating at 120 °C. Acetonitrile and pyridine were purified according to the standard procedures and freshly distilled prior to use. All other reagents used were obtained from commercial sources and were of the highest grade available.

Plant materials: Achenes of C. sinica were collected in the Weinan region of Shaanxi Province, in April 2002 and were authenticated by Prof. Z. H. Wu from the Northwest Institute of Botany, Yangling, Shaanxi, China. A voucher specimen (LXY 00016) is deposited at the Natural Medicine Resource Centre, College of Sciences, Northwest A&F University, Yangling, China.

Extraction and isolation. The dried and powdered achenes (30 Kg) of *C. sinica* were extracted with 95% EtOH three times at rt. The resulting extract was suspended in water and partitioned with petroleum ether and $CHCl_3$. The $CHCl_3$ was subjected to silica gel CC with petroleum ether-AcOEt (95:5, 15:1, 90:10, 80:20, 70:30, 60:40, 50:50, 30:70, 10:90) to provide six fractions (fr. A-F). Fr. D was rechromatographed over silica gel column eluting with petroleum ether-acetone (20:1, 15:1, 10:1, 8:2, 6:4, 5:5, 3:7, 9:1) to give five subfractions (subfr. G-K). Subfr G containing a mixture of compounds **1** and **3** was further separated preparative Waters 600E HPLC [elutent: 14% MeCN in water, λ=215 nm, flow rate= 5 mL/min, retention time= 6.70 (major) and 11.29 (minor) min] to afford two isolates **1** and **3** in pure form. Subfr I was further purified by silica gel CC using petroleum ether-acetone (6:4), yielding compound **2.**

Tutin (1). Colorless cubic crystals, mp 206-207 °C (Ref.,[1c] 205-207 °C); IR(KBr) ν= 3530(OH), 3000, 1760(C=O), 1654(C=C), 1468, 1165 cm^{-1}; ^1H NMR in acetone-d_6: δ ppm 1.30 (s, 3H, H-7), 1.91 (s, 3H, H-10), 2.59 (d, J = 6.1 Hz, 1H, H-14), 3.01 (d, J = 3.1 Hz, 1H, H-12), 3.10 (d, J = 4.3Hz, 1H, H-5), 3.22 (brs, 1H, H-4), 3.64 (d, J = 3.1Hz, 1H, H-11), 3.94 (d, J = 6.0 Hz, 1H, H-14), 4.20 (s, 1H, H-2), 4.65 (s, 1H, H-9), 4.81 (s, 1H, H-3), 4.82 (s, 1H, H-9); ^{13}C NMR in acetone-d_6: δ ppm 45.6 (C-1), 78.0 (C-6), 84.1 (C-3), 50.0 (C-4), 50.3 (C-5), 72.6 (C-2), 22.9 (C-7), 143.1 (C-8), 110.6 (C-9), 20.7(C-10), 60.8 (C-11), 60.1 (C-12), 65.8 (C-13), 51.8 (C-14),175.4 (C-15); ESIMS *m/z* 295.29 $[M+H]^+$. NMR data were in agreement with the reported data for tutin.[1b-d]

Coriatin (2). Colorless crystals, mp 259-262 °C (Ref.,[5] 263-265 °C); IR(KBr) ν= 3270(OH), 3080, 2970, 1774(OC=O), 1445 cm^{-1} ; ^1H NMR in acetone-d_6: δ ppm 1.05 (s, 3H, H-7), 1.35 (s, 3H, H-10), 1.44 (s, 3H, H-9), 1.57(dd, J = 3.68, and 3.77 Hz, 1H, H-2), 1.96 (d, J = 15.1 Hz, 1H, H-2), 2.48 (m, 1H, H-4), 2.76(d, J = 4.17 Hz, 1H, H-14), 2.98 (d, J = 4.73 Hz, 1H, H-5), 3.00 (d, J = 4.23 Hz, 1H, H-14), 3.13 (d, J = 3.02 Hz, 1H, H-12), 3.67 (d, J = 3.02 Hz, 1H, H-11), 4.82 (t, J = 4.34 Hz, 1H, H-3); ^{13}C NMR in acetone-d_6: δ ppm 40.0 (C-1), 32.1 (C-2), 78.8 (C-3), 49.7 (C-4), 52.6 (C-5), 75.7 (C-6), 22.4 (C-7), 69.3 (C-8), 30.1 (C-9), 28.5 (C-10), 61.3 (C-11), 58.5 (C-12), 67.1 (C-13), 52.1 (C-14), 174.9 (C-15). ESIMS *m/z* 297.31 $[M+H]^+$. NMR data were in agreement with the reported data for coriatin.[1c,5]

Crystal data for tutin (1) and coriatin (2). All measurements were made on a Bruker Smart 1000 CCD system diffractometer with graphite monochromated Mo Kα radiation λ=0.71073 Å at 291 K. Crystal data for **1**: $C_{15}H_{18}O_6$, M=294.29, orthorhombic, space group $P2_12_12_1$ with cell constants a = 7.5458(15) (Å), b = 12.333(3) (Å), c = 14.582(3) (Å), α = 90º, β = 90º, γ = 90º, V = 1357.0(5) $Å^3$, Z=4, D_{calcd}

=1.440 g/cm^3, m = 0.112 mm^{-1} and F(000)=624. Crystal size: 0.20 × 0.18 × 0.18 mm^3. The total number of independent reflections measured was 4203, of which 2314 were unique [R(int) = 0.0225]; crystal data for **2**: $C_{15}H_{20}O_6$, M=296.31, orthorhombic, space group $P2_12_12_1$ with cell constants a = 6.9190(14) (Å), b = 13.703(3) (Å), c = 14.886(3) (Å), $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, V = 1411.4(5) Å^3, Z=4, D_{calcd} =1.394 g/cm^3, m = 0.108 mm^{-1} and F(000)=632. Crstal size: 0.20 × 0.18 × 0.17 mm^3. The total number of independent reflections measured was 4495, of which 2467 were unique [R(int) = 0.0435]. The structure was solved by direct methods (SHELXS-97)[6] and refined using full matrix least-squares difference Fourier techniques. All nonhydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. The final agreement factors of R_1 = 0.0317 and wR_2 = 0.0727 [I>2 σ (I)] for **1,** and R_1 = 0.0364 and wR_2 = 0.0839 [I>2 σ (I)] for **2**.

Corianin (3). Colorless crystals, mp 216-217 °C (Ref.,[4] 215-216 °C); IR(KBr) ν= 3270 (OH), 3080, 2970, 1774 (OC=O), 1445 cm^{-1}; ^{1}H NMR in acetone-d_6: δ ppm 1.07 (s, 3H, H-7), 1.95 (s, 3H, H-10), 3.13 (d, J = 4.0 Hz, 1H, H-5), 3.37 (m, 1H, H-4), 3.49 (d, J = 2.4 Hz, 1H, H-11), 3.78 (d, J = 2.4 Hz, 1H, H-12), 3.79, 3.88 (dd, J = 10.0 Hz, 10.0Hz, 2H, H-14), 4.21 (d, J = 4.0, 1H, H-2), 4.89, 4.92 (brs, 2H, H-9), 5.01 (t, J = 4.0 Hz, 1H, H-3); ^{13}C NMR in acetone-d_6: δ ppm 55.3 (C-1), 81.9 (C-2); 85.5 (C-3), 48.9 (C-4), 49.9 (C-5), 75.6 (C-6), 22.9 (C-7), 141.3 (C-8), 112.5 (C-9), 21.7 (C-10), 64.1 (C-11), 60.9 (C-12), 90.4 (C-13), 77.5 (C-14), 175.3 (C-15); ESIMS *m/z* 295.29 [M+H]$^+$. NMR data were consistent with the reported data for corianin.[4,5]

Preparation of acylation derivatives (1a-c) of tutin (1). To a stirred solution of tutin (20-40 mg) and acyl chloride (1mL) in AcOEt (10 mL) was added pyridine (1.5 mL) and a catalytic amount of DMAP at rt, and then the mixture was further stirred for 6 h, with monitoring by TLC analysis. The reaction mixture was poured into ice water (10 mL) and extracted with AcOEt (3×10 mL). The combined organic layer was washed with 10% aqueous $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. After evaporation *in vacuo*, the resulting solid was subjected to a silica-gel column chromatography with AcOEt-hexane (9:1, 8:2, 3:2) as the eluent to give compounds **1a-c** (Scheme **1**) in almost quantitative yield.

2-Benzoyltutin (1a). Colorless crystals, IR (KBr) ν= 3530(OH), 3000, 1760(C=O), 1654(C=C), 1606(C=C), 1468, 1165 cm^{-1}; ^{1}H NMR in acetone-d_6, see Table 1; ^{13}C NMR in acetone-d_6, see Table 2; HRESIMS *m/z* 421.1250 [M+H]$^+$ ($C_{22}H_{22}O_7$ calcd 421.1261).

2-*iso*-Butenoyltutin (1b). Colorless crystals, mp 230.5-232.4 °C; IR (KBr) ν= 3371(OH), 2924, 2856, 1748(C=O), 1709(C=O), 1635(C=C), 1457, 1406, 1328, 1165 cm^{-1}; ^1H NMR in acetone-d_6, see Table 1; ^{13}C NMR in acetone-d_6, see Table 2; HRESIMS *m/z* 385.1264$[M+H]^+$ ($C_{19}H_{22}O_7$ calcd 385.1272).

2-Chloroacetyltutin (1c). Colorless crystalline solid, mp 198.7-200.5°C; IR (KBr) ν= 3386(OH), 2999, 2957, 1753(C=O), 1641(C=C), 1410, 1376, 1322, 1182, 1066 cm^{-1}; ^1H NMR in acetone-d_6, see Table 1; ^{13}C NMR in acetone-d_6, see Table 2; HRESIMS *m/z* 393.0717$[M+H]^+$ ($C_{17}H_{19}O_7Cl$ calcd 393.0725).

Antifeedant test. The antifeeding activity of the tutin **1** and semisynthetic compounds **1a–c** was assayed by presenting them on leaf disks of a corn against the fourth instar larvae of *Mythimna separate* (Walker) using a non-choice leaf disk method at a concentration of 2mg/mL in acetone, and visually comparing the treated and untreated disks as control eaten by the larvae. The activity was expressed as percentage of feeding inhibition, antifeedant rate (%)=[(C-T)/C] ×100, where C is the consumption of control disks and T the consumption of treated disks.

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