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ANTIFEEDANT ACTIVITIES OF TUTIN AND ANDROGRAPHOLIDE DERIVATIVES AGAINST *MYTHIMNA SEPARATA*

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Abstract – Nine andrographolide derivatives (**A2, A3a-b, A4a-e, A5**) were synthesized to study the antifeedant activities of these derivatives and tutin against *Mythimna separata*. Compared with tutin, the nine derivatives exhibited better antifeedant activities, and six of them showed different mortality effects. It was concluded that the potent insecticidal agents are **A2** (14-deoxy-11,12-didehydro-andrographolide), **A4d** (15-fluorobenzylidene-14-deoxy-11,12-didehydro-andrographolide) and **A4e** (15-bromoacylbenzylidene-14-deoxy-11,12-didehydro-andrographolide).

Tutin (Figure 1) exhibits marked antifeedant and stomach toxicity effects on many kinds of pests.[1,2] Previous results have indicated that the lactone moiety of tutin increased insecticidal activity, while the hydroxyl group at C-2 decreased activity, and the acylated analogues derived from tutin were more potent than the parent.[2] Andrographolide (**A1)** (Figure 1) is a bicyclic diterpenoid lactone that is structurally similar to tutin.[3] **A1** and its derivatives have antibacterial and cytotoxic effects.[4-7] However, no systematic study has been completed to improve the toxicities associated with insecticide **A1** and to synthesize novel and potent agents. Accordingly, we prepared andrographolide derivatives and compared their insecticidal activities with tutin, which prompted us to study andrographolide to synthesize novel potent insecticides. In this study, **A1** underwent dehydration at C-14 to afford 14-deoxy-11,12-didehydro-andrographolide

(**A2**). From **A2**, acyl derivative **A3** and alkylidene derivative **A4** were obtained through acylation at C-3 and C-19 and arylation at C-15, respectively. Additionally **A5** was obtained by acylation of **A4b**. The antifeedant activities of these derivatives and tutin were evaluated.

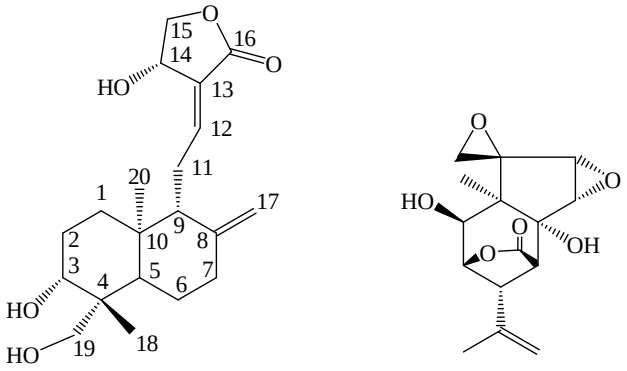
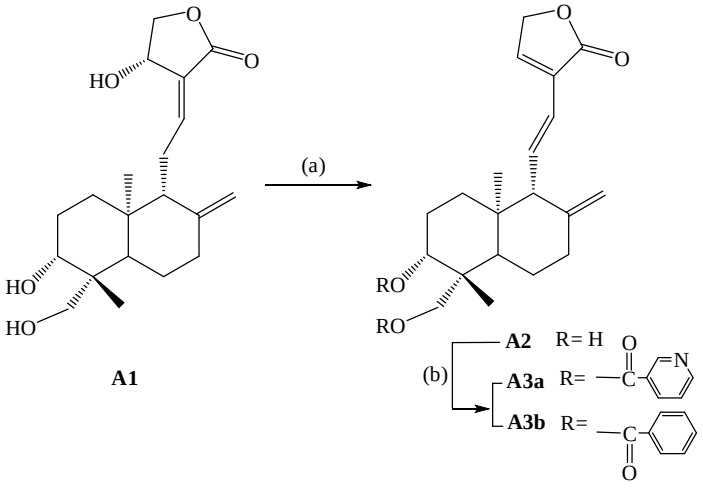


Figure 1. Structures of andrographolide (**A1**) and tutin

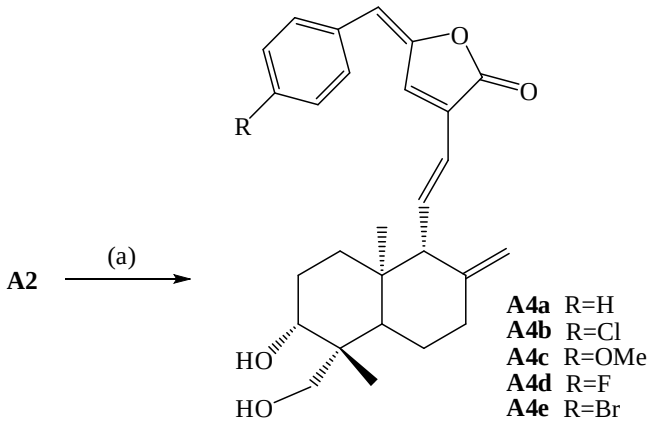
The structure of andrographolide contains an α-alkylidene-γ-butyrolactone moiety, two olefin bonds $\Delta^{8(17)}$ and $\Delta^{12(13)}$, and three hydroxyl groups at C-3, C-19 and C-14. The allylic hydroxyl at C-14 and the exocyclic double bond $\Delta^{12(13)}$ can be rearranged by adduction due to their unstable chemical properties.[8] To understand the role of the allylic hydroxyl at C-14 and the conjugated double bond, **A1** and Al_2O_3 were refluxed in dry pyridine in the presence of 4-dimethylaminopyridine (DMAP) to afford **A2**, in which the exocyclic double bond ($\Delta^{12(13)}$) isomerized to the endocyclic double bond ($\Delta^{13(14)}$) and another exocyclic double bond ($\Delta^{11(12)}$), with the simultaneous removal of the C-14 hydroxyl group. For further study, the hydroxyls at C-3 and C-19 of **A2** were acylated simultaneously. The acyl derivative **A3** was synthesized by refluxing **A2** and nicotinyl chloride or benzoyl chloride in chloroform in the presence of DMAP (Scheme 1).



Scheme 1. Reagents and conditions: (a) dry pyridine, Al_2O_3/reflux, 4 h; (b) RCOCl, DMAP, $CHCl_3$, reflux, 3 h.

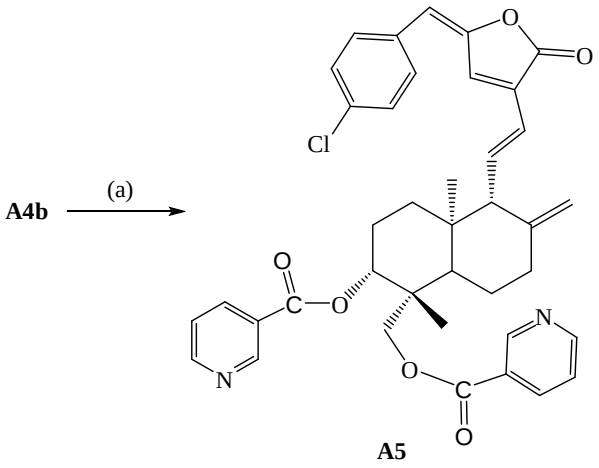
The structural assignments of synthetic compounds were examined based on their corresponding analytical and spectral data. For instance, in the ^{13}C-NMR spectrum of 14-deoxy-11,12-didehydro-3,19-dinibenzoylchloride-andrographolide (**A3b**), compound **A3b** displayed 14 more peaks corresponding to carbon atoms than did compound **A2**. The signal at 174.14 ppm, attributed to the lactone moiety of compound **A2,** still existed in **A3b**, while the signal at 168.14 ppm indicated that the two hydroxyl group of **A2** at C-3 and C-19 had been acylated simultaneously. In the ^{1}H-NMR spectrum of **A3b**, the two signals at 7.19 ppm and 2.06 ppm, corresponding to the hydroxyl groups of compound **A2,** were absent, but instead six signals ascribed to two benzene rings appeared between 7.16-8.43 ppm, which is additional evidence in support of the simultaneous acylation. Furthermore, the FT-IR spectrum of **A3b** presented different absorption bands at 1692 cm^{-1}, 758 cm^{-1} and 714 cm^{-1}, which were assignable to benzene and bands at 1707 cm^{-1}, 1744 cm^{-1} and 1287 cm^{-1}, which corresponded to ester moieties. All previously discussed analytical data, in addition to the ESIMS *m/z* of 436.78 [M]$^{+}$ and mp of 175-180 °C, strongly support the proposed acylated structure of compound **A3b**.

Because the endocyclic double bond ($\Delta^{13(14)}$) of **A2** can lead to the active secondary carbon atoms at C-15, we prepared five alkylidene andrographolide derivatives. **A4** was synthesized by refluxing **A2** with various benzaldehydes in dry MeOH in the presence of Na_2CO_3 (Scheme 2).



Scheme 2. Reagents and conditions: (a) aldehydes, Na_2CO_3, MeOH, reflux, 3-5 h.

To determine the importance of the hydroxyl groups of the alkylidene andrographolide derivatives for insecticidal activity, **A5** was prepared by acylation at C-3 and C-19 of **A4b**. The synthetic conditions to give **A5** were similar to those for **A3a**; dehydration of **A4b** in boiling CH_2Cl_2 in the presence of nicotinyl chloride and DMAP gave **A5** as the product (Scheme 3).



Scheme 3. Reagents and conditions: (a) $CHCl_3$, nicotinyl chloride, DMAP, reflux, 3 h.

Antifeedant effects of these derivatives and tutin were determined by a conventional leaf disk method against the third instar larvae of *Mythimna separata* (Walker) at 24 h and 48 h under the sublethal concentration of 2.0 mg/mL.[1] All nine derivatives showed good bioactivity, and the antifeeding rates were statistically significant ($P < 0.01$) when compared to tutin. Compared to tutin, **A2**, **A4d** and **A4e** demonstrated strong antifeeding rates, which were 116.1-125.0% and 43.1-52.4% higher at 24 h and 48 h, respectively. The test insects survived in the presence of tutin, **A3b**, **A4a** and **A4c** with the sublethal dose of 2.0 mg/mL; however, the other six derivatives exhibited different mortality effects against *Mythimna separata*. For example, **A4e** showed a mortality rate of 10.0% at 24 h, and **A4d** showed a mortality rate of 16.7% at 48 h.

Table 1. Antifeedant effects of andrographolide derivatives and tutin against 3rd instar *Mythimna separate*

Compound	**A2**	**A3a**	**A3b**	**A4a**	**A4b**	**A4c**	**A4d**	**A4e**	**A5**	Tutin
24 h-antifeeding rate (%)	50.9**	40.0*	42.5**	25.8	45.3**	27.5	51.2**	53.0**	44.4**	23.6
24 h-relative variability rate (%)	116.1	70.0	80.3	9.6	92.5	16.8	117.5	125.0	88.6	0.0
24 h-mortality rate (%)	3.3	3.3	0.0	0.0	3.3	0.0	6.7	10.0	3.3	0.0
48 h-antifeeding rate (%)	51.5**	36.8	45.0*	42.6	45.5*	37.6	54.8**	51.7**	40.7	36.0
48 h-relative variability rate (%)	43.1	2.3	25.1	18.4	26.4	4.5	52.4	43.7	13.2	0.0
48 h-mortality rate (%)	3.3	3.3	0.0	0.0	10.0	0.0	16.7	13.3	3.3	0.0

Data were analyzed using one-way ANOVA, where $^{*}P < 0.05$ and $^{**}P < 0.01$ were significantly different from tutin.

EXPERIMENTAL

General. Melting points were recorded on a XT-5 micro melting point apparatus and were uncorrected. The IR spectra were obtained on a 4300 Shimadzu spectrometer, and only noteworthy absorptions were listed. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX-500 Fourier transformer spectrometer at 500 MHz in pyridine-d5 for compounds **A4b** and **A4e** and in deuterochloroform ($CDCl_3$) for all other compounds. Chemical shifts were reported in δ (ppm) values downfield from TMS as internal standard; coupling constants (J) were given in Hertz. MS were recorded using a Q-TOF MicroTM spectrometer. Dichloromethane (CH_2Cl_2) was purified according to the standard procedures and was freshly distilled prior to use. All other reagents used were obtained from commercial sources and were of the highest grade available.

14-Deoxy-11,12-didehydro-andrographolide (A2): Al_2O_3 (1.3 g, 13.0 mmol) was added to the solution of andrographolide (8.0 g, 23.0 mmol) in dry pyridine. The mixture was refluxed for 12 h and then filtered to remove the solid Al_2O_3. The filter cake was washed with $CHCl_3$. The solvent was removed under vacuum to give crude deoxyandrographolide, which was recrystallized in MeOH to yield compound **A2** as a white powder (6.8 g, 89%). mp 207-210 °C; IR (KBr) cm^{-1}: 3477 (-OH), 3431 (-OH), 2934 (-CH_3), 1737 (-C=O), 1635 (C=C), 1448, 1437, 1401, 1352; ESIMS *m/z* 331.42 $[M-H]^+$. NMR data were in agreement with the reported data for **A2**.[9]

14-Deoxy-11,12-didehydro-3,19-dinicotinate-andrographolide (A3a): Compound **A2** (1.0 g, 3.0 mmol) and nicotinyl chloride (1.6 g, 11.4 mmol) in $CHCl_3$ were refluxed for 3 h under Ar in the presence of Na_2CO_3 and DMAP. The reaction mixture was extracted with Na_2CO_3 (aq), brine, and water successively. The $CHCl_3$ phase was dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was recrystallized from MeOH to give **A3a** as a beige powder (1.47 g, 90%). mp 225-228 °C; IR (KBr) cm^{-1}: 1744 (-C=O), 1707 (-C=O), 1418, 1326 (C-N), 1298 (C-N); ESIMS *m/z* 543.02 $[M+H]^+$. NMR data were in agreement with the reported data for **A3a**.[10]

14-Deoxy-11,12-didehydro-3,19-dinibenzoylchloride-andrographolide (A3b): white powder, mp 175-180 °C; IR (KBr) cm^{-1}: 1744 (-C=O), 1692 (-ph), 1338 (O-H), 1317, 1287, 1131, 1086 (-OH), 1048, 758 (-C_6H_5), 714 (-C_6H_5); ^{1}H-NM ($CDCl_3$): δ 1.24 (m, 2H, *J* = 6.0 Hz, H-1), δ 1.90 (dd, 2H, *J* = 3.5 Hz, H-2), δ 3.64 (m, 1H, *J* = 5.3 Hz, H-3), δ 1.55 (m, 1H, *J* = 5.5 Hz, H-5), δ 1.32(m, 2H, *J* = 6.8 Hz, H-6), δ 2.04 (m, 2H, *J* = 8.7 Hz, H-7), δ 1.79 (m, 1H, *J* = 8.5 Hz, H-9), δ 4.84 (m, 1H, *J* = 11.0 Hz, H-11), δ 5.07 (d, 1H, *J* = 11.5 Hz, H-12), δ 6.29 (d, 1H, *J* = 16.0 Hz, H-14), δ 4.79 (s, 2H, H-15), δ 4.80 (s, 1H, H-17a), 4.76 (s, 1H, H-17b), δ 1.02 (s, 3H, H-18), δ 2.45 (m, 2H, *J* = 7.5 Hz, H-19), δ 1.51 (s, 3H, H-20), δ 8.28

(m, 2H, *J* = 4.2 Hz, H-23, 27), *δ* 7.52 (t, 2H, *J* = 3.8 Hz, H-24, 26), *δ* 7.48 (t, 1H, *J* = 9.2 Hz, H-25), *δ* 7.16 (m, 1H, *J* = 7.7 Hz, H-30), *δ* 7.43 (t, 2H, *J* = 7.5 Hz, H-31, 33), *δ* 7.33 (s, 2H, H-32), *δ* 8.43 (d, 1H, *J* = 7.5 Hz, H-34); ^{13}C-NMR ($CDCl_3$): *δ* 38.51 (C-1), 24.62 (C-2), 71.61 (C-3), 40.27 (C-4), 44.63 (C-5), 26.22 (C-6), 55.77 (C-7), 150.51 (C-8), 63.19 (C-9), 40.55 (C-10), 146.33 (C-11), 123.35 (C-12), 131.50 (C-13), 134.44 (C-14), 79.36 (C-15), 174.14 (C-16), 110.10 (C-17), 16.66 (C-18), 67.91 (C-19), 29.87 (C-20), 168.14 (C-21, 28), 130.01 (C-22, 29), 131.20 (C-23, 27, 30, 34), 130.18 (C-24, 26, 31, 33), 132.70 (C-25, 32); ESIMS *m/z* 436.78 $[M]^+$.

15-Benzylidene-14-deoxy-11,12-didehydro-andrographolide (A4a): Compound **A2** (1.0 g, 3.0 mmol) and various aldehydes or ketones (4.5-9.0 mmol) were refluxed in dry MeOH in the presence of Na_2CO_3 (100 mg, 0.9 mmol). After completion of the reaction, the mixture was diluted with $CHCl_3$ and washed with water. The organic phase was evaporated in vacuo to afford the corresponding product, which was purified by flash chromatography or crystallization from MeOH to give the pure product as a beige powder (1.1 g, 87%), mp 210-215 °C; IR (KBr) cm^{-1}: 3265 (-OH), 2931, 1754 (-C=O), 1642 (C=C), 1451, 1375, 1361, 1323, 1298, 1282; ESIMS *m/z* 420.69 $[M]^+$. NMR data were in agreement with the reported data for **A4a**.[10]

15-Chlorobenzylidene-14-deoxy-11,12-didehydro-andrographolide (A4b): beige powder, mp 237-240 °C; IR (KBr) cm^{-1}: 3269 (-OH), 177 0 (-C=O), 1754, 1710, 1489, 1447, 1412, 1360, 1280, 1091; ^{1}H-NMR (pyr-d5): *δ* 1.49 (m, 2H, *J* = 3.0 Hz, H-1), *δ* 2.03 (dd, 2H, *J* = 8.5 Hz, H-2), *δ* 1.95 (dd, 1H, *J* = 7.8 Hz, H-3), *δ* 1.16 (t, 1H, *J* = 6.7 Hz, H-5), *δ* 1.41 (dd, 1H, *J* = 5.7 Hz, H-6a), *δ* 1.22 (dd, 1H, *J* = 10.0 Hz, H-6b), *δ* 2.42 (t, 2H, *J* = 8.5 Hz, H-7), *δ* 1.79 (m, 1H, *J* = 4.3 Hz, H-9), *δ* 7.19 (d, 1H, *J* = 7.7 Hz, H-11), *δ* 4.48 (d, 1H, *J* = 11.0 Hz, H-12), *δ* 6.38 (d, 1H, *J* = 16.0 Hz, H-14), *δ* 4.88 (s, 1H, H-17a), *δ* 4.75 (s, 1H, H-17b), *δ* 0.90 (s, 3H, H-18), *δ* 3.66 (d, 2H, *J* = 10.5 Hz, H-19), *δ* 1.52 (s, 3H, H-20), *δ* 6.13 (s, 1H, H-21), *δ* 7.38 (d, 2H, *J* = 8.5 Hz, H-23, 27), *δ* 7.75 (d, 2H, *J* = 8.5 Hz, H-24, 26); ^{13}C-NMR (pyr-d5): *δ* 36.78 (C-1), 28.7 (C-2), 79.9 (C-3), 38.6 (C-4), 43.2 (C-5), 23.5 (C-6), 54.5 (C-7), 148.5 (C-8), 61.8 (C-9), 39.0 (C-10), 138.2 (C-11), 121.8 (C-12), 132.6 (C-13), 136.3 (C-14), 168.8 (C-16), 108.8 (C-17), 15.9 (C-18), 64.0 (C-19), 23.4 (C-20), 111.6 (C-21), 127.4 (C-22), 129.1 (C-23, 27), 131.9 (C-24, 26); ESIMS *m/z* 455.99 $[M+H]^+$.

15-Methoxylbenzylidene-14-Deoxy-11,12-didehydro-andrographolide (A4c): bright yellow powder, mp 209-214 °C (Ref. 1c: 205-207 °C); IR (KBr) cm^{-1}: 3277 (-OH), 2926, 2851, 1742 (-C=O), 1640 (C=C), 1511, 1454, 1304, 1256, 1180; ^{1}H-NMR ($CDCl_3$): *δ* 1.76 (t, 1H, *J* = 4.0 Hz, H-1), *δ* 1.82 (dd, 2H, *J* = 4.5 Hz, H-2), *δ* 3.50 (m, 1H, *J* = 7.5 Hz, H-3), *δ* 7.12 (s, OH, H-3), *δ* 1.18 (t, 2H, *J* = 13.8 Hz, H-5), *δ*

1.38 (dd, 2H, *J* = 5.7 Hz, H-6), *δ* 2.48 (d, 1H, *J* = 14.0 Hz, H-7a), *δ* 2.37 (d, 1H, *J* = 10.0 Hz, H-7b), *δ* 1.55 (d, 1H, *J* = 13.5 Hz, H-9), *δ* 6.20 (d, 1H, *J* = 15.5 Hz, H-11), *δ* 6.93 (dd, 1H, *J* = 8.0 Hz, H-12), *δ* 7.28 (s, 1H, H-14), *δ* 4.81 (s, 1H, H-17a), *δ* 4.57 (s, 1H, H-17b), *δ* 0.85 (s, 3H, H-18), *δ* 4.24 (d, 2H, *J* = 11.0 Hz, H-19), *δ* 1.28 (s, 3H, H-20), *δ* 6.66 (s, 1H, H-21), *δ* 7.34 (d, 2H, *J* = 8.5 Hz, H-23, 27), *δ* 7.74 (d, 2H, *J* = 8.5 Hz, H-24, 26), *δ* 1.27 (s, 3H, H-28); ^{13}C-NMR ($CDCl_3$): *δ* 36.6 (C-1), 23.0 (C-2), 80.8 (C-3), 43.0 (C-4), 54.7 (C-5), 28.1 (C-6), 38.3 (C-7), 148.0 (C-8), 62.0 (C-9), 38.8 (C-10), 135.6 (C-11), 121.6 (C-12), 126.3 (C-13), 138.3 (C-14), 146.3 (C-15) , 169.1 (C-16), 109.3 (C-17), 16.0 (C-18), 64.2 (C-19), 22.7 (C-20), 113.2 (C-21), 132.2 (C-22), 130.5 (C-23, 27), 130.8 (C-24, 26), 129.6 (C-25); ESIMS *m/z* 450.76 $[M]^+$.

15-Fluorobenzylidene-14-deoxy-11,12-didehydro-andrographolide (A4d): bright yellow powder, mp 202-207 °C; IR (KBr) cm^{-1}: 3291 (-OH), 2929, 1743 (-C=O), 1644 (C=C), 1599, 1506, 1440, 1362, 1291, 1229, 1163, 1082, 1033; ^{1}H-NMR ($CDCl_3$): *δ* 1.35 (m, 2H, *J* = 4.0 Hz, H-1), *δ* 1.82 (t, 2H, *J* = 12.5 Hz, H-2), *δ* 1.75 (dd, 1H, *J* = 4.2 Hz, H-3), *δ* 1.19 (t, 1H, *J* = 5.7 Hz, H-5), *δ* 1.24 (dd, 2H, *J* = 8.7 Hz, H-6), *δ* 2.49 (d, 1H, *J* = 13.5 Hz, H-7), *δ* 2.38 (d, 1H, *J* = 10.5 Hz, H-7b), *δ* 1.57 (t, 1H, *J* = 3.0 Hz, H-9), *δ* 6.93 (dd, 1H, *J* = 8.7 Hz, H-11), *δ* 4.24 (d, 1H, *J* = 11.0 Hz, H-12), *δ* 6.22 (d, 1H, *J* = 15.5 Hz, H-14), *δ* 4.82 (s, 1H, H-17a), *δ* 4.56 (s, 1H, H-17b), *δ* 0.86 (s, 3H, H-18), *δ* 3.42 (m, 2H, *J* = 5.0 Hz, H-19), *δ* 1.29 (s, 3H, H-20), *δ* 5.94 (s, 1H, H-21), *δ* 7.11 (m, 2H, *J* = 7.8 Hz, H-23, 27), *δ* 7.78 (m, 2H, *J* = 4.8 Hz, H-24, 26); ^{13}C-NMR ($CDCl_3$): *δ* 36.59(C-1), 22.99(C-2), 80.86 (C-3), 38.34 (C-4), 43.05 (C-5), 22.66 (C-6), 54.72 (C-7), 148.05 (C-8), 61.98 (C-9), 38.80 (C-10), 137.80 (C-11), 121.46 (C-12), 127.04 (C-13), 135.40 (C-14), 147.24 (C-15), 168.73 (C-16), 109.33 (C-17), 15.96 (C-18), 64.19 (C-19), 28.14 (C-20), 111.80 (C-21), 129.66 (C-22), 115.90 (C-23), 132.31 (C-24), 163.84 (C-25), 132.38 (C-26), 116.07 (C-27); ESIMS *m/z* 461.67 $[M+Na]^+$.

15-Bromoacylbenzylidene-14-deoxy-11,12-didehydro-andrographolide (A4e): white powder, mp 240-244 °C; IR (KBr) cm^{-1}: 3275 (-OH), 2928, 1771 (-C=O), 1754, 1280, 1075, 1035, 1009, 973, 942, 904, 895; ^{1}H-NMR (pyr-d5): *δ* 1.50 (m, 2H, *J* = 5.7 Hz, H-1), *δ* 2.03 (m, 2H, *J* = 4.7 Hz, H-2), *δ* 1.90 (m, 1H, *J* = 13.2 Hz, H-3), *δ* 1.16 (m, 1H, *J* = 4.0 Hz, H-5), *δ* 1.42(dd, 1H, *J* = 5.8 Hz, H-6a), *δ* 1.20 (dd, 1H, *J* = 7.2 Hz, H-6b), *δ* 2.42 (m, 2H, *J* = 4.3 Hz, H-7), *δ* 1.78 (m, 1H, *J* = 2.5 Hz, H-9), *δ* 7.23 (t, 1H, *J* = 7.7 Hz, H-11), *δ* 4.49 (d, 1H, *J* = 11.0 Hz, H-12), *δ* 6.39 (m, 1H, *J* = 15.5 Hz, H-14), *δ* 4.89 (d, 1H, *J* = 1.0 Hz, H-17a), *δ* 4.76 (d, 1H, *J* = 1.0 Hz, H-17b), *δ* 0.91 (s, 3H, H-18), *δ* 3.68 (m, 2H, *J* = 5.5 Hz, H-19), *δ* 1.53 (s, 3H, H-20), *δ* 6.12 (s, 1H, H-21), *δ* 7.54 (m, 2H, *J* = 5.8 Hz, H-23, 27), *δ* 7.54 (d, 2H, *J* = 5.8 Hz, H-24, 26); ^{13}C-NMR (pyr-d5): *δ* 38.28 (C-1), 24.99 (C-2), 81.38 (C-3), 40.09 (C-4), 44.70 (C-5), 24.94 (C-6), 56.04 (C-7), 150.46 (C-8), 63.33 (C-9), 40.54 (C-10), 139.74 (C-11), 123.35 (C-12), 128.98 (C-13),

137.81 (C-14), 150.12 (C-15), 170.29 (C-16), 110.24 (C-17), 17.37 (C-18), 65.53 (C-19), 30.20 (C-20), 113.03 (C-21), 124.38 (C-22), 133.61 (C-23, 27), 134.49 (C-24, 26); ESIMS *m/z* 500.44 $[M+H]^+$.

15-Chlorobenzylidene-14-deoxy-11,12-didehydro-3,19-dinicotinate-andrographolide (A5): bright orange powder, mp 217-223 °C; IR (KBr) cm^{-1}: 1770 (-C=O), 1711, 1591 (C=C), 1321, 1297, 1275, 1127, 738, 699; ^{1}H-NMR ($CDCl_3$): δ 1.54 (d, 2H, J = 2.0 Hz, H-1), δ 1.91 (m, 2H, J = 3.8 Hz, H-2), δ 5.02 (d, 1H, J = 8.0 Hz, H-3), δ 4.86 (d, 1H, J = 11.5 Hz, H-5), δ 1.70 (m, 2H, J = 7.2 Hz, H-6), δ 2.49 (d, 2H, J = 10.0 Hz, H-7), δ 1.42 (d, 1H, J = 7.0 Hz, H-9), δ 7.01 (m, 1H, J = 7.7 Hz, H-11), δ 7.15 (d, 1H, J = 7.0 Hz, H-12), δ 7.28 (s, 1H, H-14), δ 4.62 (s, 1H, H-17a), δ 4.59 (s, 1H, H-17b), δ 1.03 (s, 3H, H-18), δ 2.14 (s, 1H, H-19a), 2.01 (s, 1H, H-19b), δ 1.27 (s, 3H, H-20), δ 8.23 (d, 1H, J = 6.0 Hz, H-23), δ 7.35 (dd, 1H, J = 6.2 Hz, H-24), δ 9.16 (d, 1H, J = 6.5 Hz, H-25), δ 8.70 (s, 1H, H-26), δ 8.22 (d, 1H, J = 1.5 Hz, H-29), δ 7.21 (m, 1H, J = 4.2 Hz, H-30), δ 8.76 (d, 1H, J = 3.5 Hz, H-31), δ 8.70 (s, 1H, H-32), δ 5.94 (s, 1H, H-33), δ 7.71 (d, 2H, J = 8.5 Hz, H-35, 39), δ 8.18 (d, 2H, J = 2.0 Hz, H-36, 39); ^{13}C-NMR ($CDCl_3$): δ 36.58 (C-1), 24.39 (C-2), 81.13 (C-3), 42.22 (C-4), 54.94 (C-5), 23.90 (C-6), 38.27 (C-7), 147.83 (C-8), 61.88 (C-9), 38.91 (C-10), 135.67 (C-11), 121.93 (C-12), 127.23 (C-13), 137.41 (C-14), 134.82 (C-15), 168.47 (C-16), 109.83 (C-17), 15.47 (C-18), 65.45 (C-19), 22.76 (C-20), 164.78 (C-21), 136.96 (C-22, 28), 137.07 (C-23, 29), 123.16 (C-24), 150.85 (C-25), 150.68 (C-26, 32), 165.23 (C-27), 123.42 (C-30), 153.41 (C-31), 111.82 (C-33), 131.83 (C-34), 131.60 (C-35, 39), 129.08 (C-36, 38); ESIMS *m/z* 664.84 $[M-H]^+$.

Pesticide activity test. The pesticide activities of semisynthetic compounds and tutin were determined by presenting them on leaf disks of wheat against the third instar larvae of *Mythimna separata* (Walker) using a non-choice leaf disk method at a concentration of 2.0 mg/mL in acetone, with acetone as the control.[11]

Thirty larvae were used for each treatment, and the entire experiment was repeated twice. The activity was expressed as percentage of feeding inhibition[12] and was calculated according to the following equation: antifeeding rate (%) = [(C-T)/C] × 100, where C is the consumption of the control disk, and T is the consumption of the treated disk. The difference in the antifeeding rate between semisynthetic compounds and tutin was expressed as the relative variability rate and was calculated according to the following equation: relative variability rate (%) = [(Xi-Xt)/Xt] × 100, where Xi is the antifeeding rate of semisynthetic compounds, and Xt is the antifeeding rate of tutin.

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