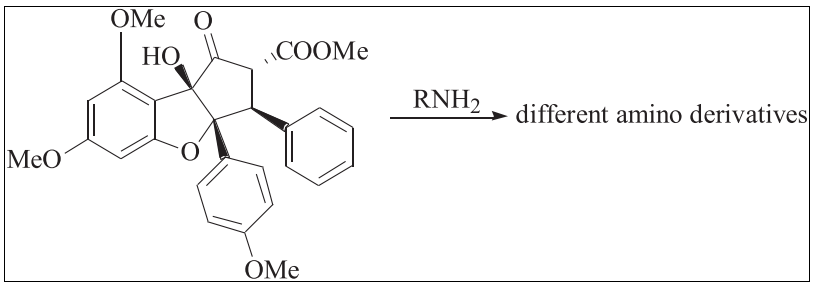
Xianyou Wang,[b] Zhuoqun Xie,[a] Bin Fu,[a]* Nan Li,[a] Mingan Wang,[a] Yongqiang Ma,[a] Fengpei Du,[a] Yanjun Xu,[a] and Zhaohai Qin[a]*

[a]College of Science, China Agricultural University, Beijing, 100193, China
[b]College of Quality and Technical Supervision, Hebei University, Baoding, 071002, China
*E-mail: fubinchem@cau.edu.cn; qinshaohai@263.net

Received February 22, 2012
DOI 10.1002/jhet.1842
Published online 25 March 2014 in Wiley Online Library (wileyonlinelibrary.com).



The synthesis of the key intermediate of rocaglamide, oxidative aglafolin, was studied, and its diastereoisomers were obtained. The amination of oxidative aglafolin was also investigated, affording amino derivatives. The preliminary bioassay results indicate that these new aglafolin derivatives showed certain degree of insecticidal and repellent activity against *Plutella xylostella* and *Laphygma exigua*.

J. Heterocyclic Chem., **51**, 1430 (2014).

INTRODUCTION

Rocaglamide (Fig. 1), featuring a cyclopenta[*b*]benzofuran core, belongs to a class of natural product possessing diverse biological activity such as insecticidal, antifungal, and antitumor activity [1]. Because of these features, rocaglamide can serve as a viable lead for the discovery of new drugs and agrochemicals.

In most rocaglamide derivatives, the phenyl groups at positions **3** and **3a** always occupy a cis orientation with respect to the hydroxyl group at position **8b**, whereas the substituents at positions **1** and **2** are in trans with respect to the hydroxyl at position **8b**. Because of the shortage of analogs with varied configurations, it is difficult to evaluate the relationship between the biological activities and different configurations. On the other hand, it appears that the 1-position carbon atom has little influence on the insecticidal activity. For example, compound **8** shows excellent insecticidal activity against littoralis [2]. Moreover, aglaiastatin and dehydro-aglaiastatin with cyclopentane fused other cycle and show almost the same good insecticidal activity, indicating that the substituents at 1-position and 2-position have little effect on the insecticidal activity, which has been ever regarded as the major site for structural modification [3,4]. Owing to oxidative aglafolin containing β-keto ester unit, further structural modification and derivatization become much more easily. We have chosen oxidative aglafolin as the key intermediate for derivatization and structure activity relationship analysis.

RESULTS AND DISCUSSION

Oxidative aglafolin (**3a**) and its isomers were synthesized according to our previously reported method (Scheme 1) [5]. Two diastereoisomers of **2a** (cis) and **2b** (trans) (the ratio of 2.24 to 1) were afforded via Michael addition of benzylidene malonate to **1** in the presence of $Bu_4N^+OH^-$.The structure of **2a** had been confirmed by X-ray diffraction analysis [6]. Subsequently, oxidative aglafolin **3** was successfully prepared from **2** via intramolecular keto-ester reductive coupling. In this process, SmI_2 was proved to be the best reductive coupling system after many trials, despite that the $Ti^{3+}$and Zr^{3+} were also used in this reduction reaction [7,8]. Through further optimizing, the solvent and other conditions, four isomers of oxidative aglafolin **3** were completely obtained, that is, **2a** converting into **3a** and **3b**, **2b** converting into **3c** and **3d**. This result is a little different from our former report; there are only two diastereoisomers because of the plausible racemerization of 2-position chiral carbon resulted from keto-ester equilibrium in compound **3**. However, herein, two couples of diastereoisomers between **3a** and **3b**, and **3c** and **3d** were successfully separated (Scheme 1).

With the precursors of rocaglamide in hand, the derivatization of β-keto ester **3** was investigated next. The amination of oxidative aglafolin **3a** with various amine compounds was studied (Scheme 2). When **3a** was reacted with hydrazine hydrate, the product pyrazolinone **4** with a fused five-membered heterocycle was given in good yield. This result can be attributed to the double condensation

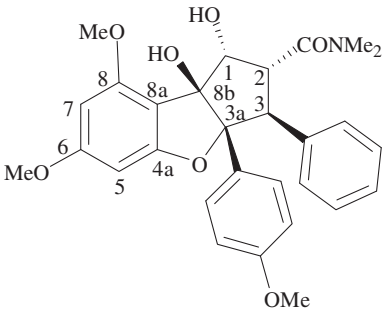
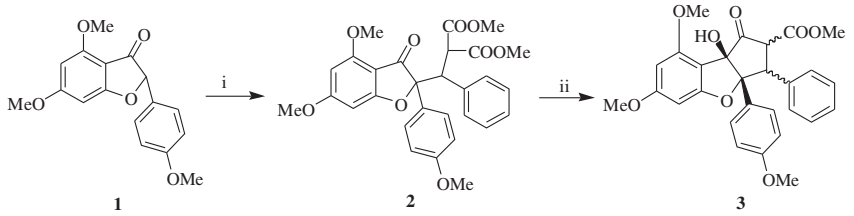


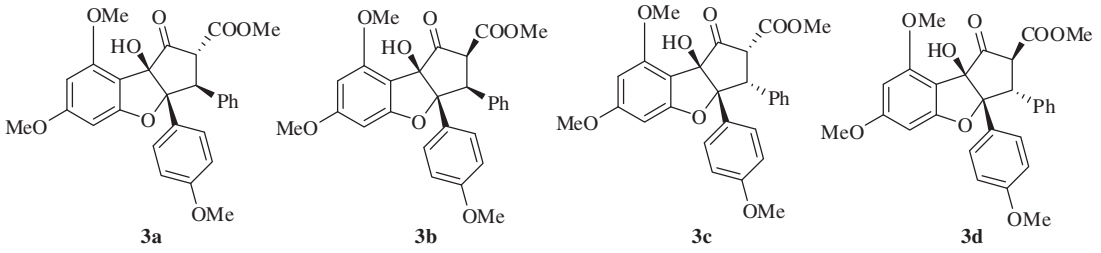
Figure 1 Rocaglamide

that occurred. Condensation of benzene hydrazine with **3a** afforded only the expected hydrazone **5** in good yield. To our surprise, the reaction of **3a** with hydroxylamine hydrochloride was more complex. Despite many trials that were attempted under various bases, such as Na_2CO_3, triethylamine, pyridine, and NaOH, the expected product **7** was not obtained even after heating at reflux. However, without the addition of base, the reaction mixture was directly refluxed in anhydrous methanol; the unexpected product **6** with a three-membered spiro-ring containing N-O bond was obtained in 61% yield. Its structure was

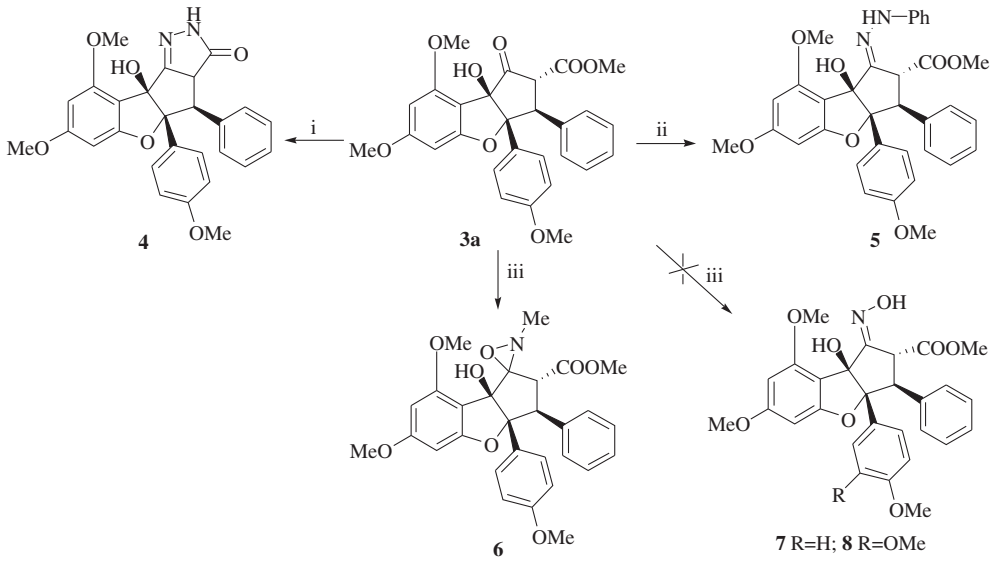
Scheme 1



Reaction condition: i.$PhCH{=}CHCO_2Me$, $Bu_4N^+OH^-$; ii. SmI_2



Scheme 2



Reaction condition: i. $NH_2NH_2.HCl$; ii) $PhNH_2NH_2$; iii) $NH_2OH.HCl$

Table 1

Insecticidal activities of the title compounds (% mortality).

		Plutella xylostella			*Laphygma exigua*		
No.	Concentration (μg/mL)	2 days	3 days	5 days	2 days	3 days	5 days
3a	100	5.0	—	47.4	15.0	40.0	70.0
	200	60.0	68.4	73.7	30.0	45.0	80.0
3b	100	5.0	42.1	57.9	0	5.0	75.0
	200	60.0	73.7	89.5	0	10.0	75.0
3c	100	0	10.5	57.9	20.0	25.0	70.0
	200	45.0	52.6	57.9	15.0	60.0	100.0
3d	100	25.0	—	52.6	0	5.0	15.0
	200	55.0	57.9	100.0	0	10.0	100.0
4	100	5.0	—	52.6	0	15.0	50.0
	200	10.0	26.3	73.7	5.0	15.0	75.0
5	100	5.0	—	63.2	0	5.0	25.0
	200	20.0	36.8	68.4	10.0	10.0	60.0
6	100	10.0	36.8	68.4	0	0	0
	200	55.0	73.7	100.0	5.0	5.0	50.0
Azadirachtin	100	10.0	5.3	100.0	30.0	55.0	100.0
	200	25.0	42.1	100.0	40.0	50.0	100.0

confirmed by ^{1}H NMR, ^{13}C NMR, IR, and HRMS. Similar products were not observed when methanol was used in place of ethanol or benzyl alcohol. The mechanism for this reaction is under further investigation.

Finally, we evaluated the insecticidal and repellent activity of all the newly synthesized compounds and compared them with azadirachtin as shown in Tables 1 and 2. The biological activities of different isomers of oxidative aglafolin **3** showed somewhat differences from the bioassay data. Especially, under the concentration of 200 μg mL^{-1}, the compound **3d** displayed 100% of insecticidal activity against *Plutella xylostella* and *Laphygma exigua*. In the test of repellency, **3c** and **3d** showed almost the same repellent as that of azadirachtin, whereas the amino- derivatives **4**, **5**, and **6** have low repellent activity against *L. exigua*. These results indicated the change of the configuration at 2-position and 3-position of oxidative aglafolin that had an important effect on the insecticidal and repellent activity.

In summary, oxidative aglafolin and its isomers were prepared; further derivatization with various amines was studied. In addition, the biological activity of its stereo-isomers and corresponding derivatives were investigated. Our research demonstrated that the configuration of oxidative aglafolin exhibited different degree of impact on the insecticidal and repellent activity. Further studies are in progress in our laboratory.

Table 2

Repellency of the title compounds against *Laphygma exigua*.

No.	Repellency (%)
Azadirachtin	50.0
3a	25.0
3b	20.0
3c	50.0
3d	45.0
4	30.0
5	0
6	0

EXPERIMENTAL

Chemistry. Melting points were measured with an XT-4 melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and are uncorrected. NMR spectra were recorded with a Bruker Avance DPX300 spectrometer (Bruker, Swiss) with tetramethylsilane as the internal standard. Mass spectra were obtained with a VG-ZAB-HS mass spectrometer (Micromass Co. UK). Solvents used were purified and dried by standard procedures. Compound **1** was synthesized according to literature procedure [5].

Synthesis of 2. Under a N_2 atmosphere, to a solution of **1** (3.0 g, 10 mmol) in anhydrous THF (100 mL) was added a solution of Triton B (40% in CH_3OH, 0.30 mL) and a solution of dimethyl benzylidene malonate (3.2 g, 14.5 mmol) in THF (30 mL) by syringe. After stirring for 3 h at 60°C, the solvent was removed in vacuo and to the residue was added a solution of HCl (1 M, 30 mL), and this solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with brine (2 × 30 mL), dried with Na_2SO_4, and concentrated. The crude product was separated by silica-gel column chromatography (petroleum ether/ethyl acetate, 1:1) to afford a couple of diastereoisomers **2a** and **2b**.

2a (Cis), 1.82 g, 35.0% yield, mp: 169–170°C. ^{1}H NMR ($CDCl_3$): δ 3.17 (s, 3H), 3.27 (s, 3H), 3.65 (s, 3H), 3.77 (s, 3H), 3.86 (s, 3H), 4.33 (d, 1H, J = 10.8 Hz), 4.53 (d, 1H, J = 10.8 Hz), 5.78 (d, 1H, J = 1.8 Hz), 6.27 (d, 1H, J = 1.8 Hz), 6.82–6.85 (m, 2H), 7.05–7.13 (m, 3H,), 7.31–7.34 (m, 2H), 7.69–7.76 (m, 2H). ^{13}C NMR δ: 194.3, 174.0, 169.5, 167.6, 167.5, 159.6, 159.1, 135.4, 130.0, 127.7, 127.5, 127.3, 127.0, 113.4, 103.7, 92.8, 92.3, 88.5, 55.8,

55.7, 55.2, 54.1, 52.2, 52.1, 51.4. IR (cm^{-1}): 2953, 1746, 1707, 1620, 1594, 1510, 1255, 1219, 1156, 1124, 841, 816, 698.

2b (Trans): 0.85 g, 16.3% yield, mp 168–169°C. ^{1}H NMR ($CDCl_3$): δ 3.29 (s, 3H), 3.54 (s, 3H), 3.67 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 4.21 (d, 1H, J = 9.3 Hz), 4.47 (d, 1H, J = 9.3 Hz), 5.97 (d, 1H, J = 1.8 Hz), 6.27 (d, 1H, J = 1.8 Hz), 6.63–6.67 (m, 2H), 7.04–7.11(m, 3H), 7.17–7.21 (m, 2H), 7.36–7.42 (m, 2H). ^{13}C NMR: δ 194.5, 174.0, 169.8, 167.8, 167.6, 159.2, 159.1, 136.2, 130.2, 128.4, 127.4, 127.0, 126.9, 113.2, 104.3, 93.2, 92.7, 88.8, 55.9, 55.1, 53.4, 52.5, 52.1, 52.0. IR (cm^{-1}): 2953, 2841, 1785, 1623, 1592, 1511, 1254, 1221, 1159, 1032, 927, 818, 698.

Synthesis of 3. (1) **3a** and **3b**: To the reactant of metal Sm (1.20 g, 8.0 mmol) in a flame-dried, three-necked round-bottom flask (100 mL) equipped with a stir bar, septum, and nitrogen inlet was added a solution of $C_2H_4I_2$ (1.10 g, 3.9 mmol) in THF (7 mL). After stirring for 1 h, the solution changed to blue, and the reaction was continued for 3 h under ultrasound irradiation. Anhydrous benzene (20 mL) was then added, and the reaction was continued for an additional 2 h. A solution of **2a** (1.02 g, 1.96 mmol) in benzene (100 mL) was added, and the reaction was allowed to proceed for 10 h under ultrasound irradiation. The reaction was quenched with the addition of HCl (1 M, 20 mL), and the solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with brine (2 × 20 mL), dried with anhydrous Na_2SO_4, and concentrated. The crude product was purified by silica-gel column chromatography (petroleum ether/EtOAc, 2:3) to afford a couple of diastereoisomers **3a** and **3b**.

3a: 0.27 g, yield: 28.6%. mp 161–162°C. ^{1}H NMR ($CDCl_3$): δ 3.04 (s, 1H), 3.66 (s, 3H), 3.71 (s, 3H), 3.81 (s, 3H), 3.85 (s, 3H), 4.06 (d, 1H, J = 13.2 Hz), 4.24 (d, 1H, J = 13.2 Hz), 6.10 (d, 1H, J = 1.8 Hz), 6.35 (d, 1H, J = 1.8 Hz), 6.66–6.70 (m, 2H), 6.89–6.97 (m, 4H), 7.08–7.12 (m, 3H). ^{13}C NMR: δ 203.3, 167.2, 164.9, 161.0, 158.9, 158.6, 135.4, 129.1, 128.0, 127.9, 127.7, 127.1, 125.4, 113.2, 112.2, 106.1, 99.3, 92.9, 89.9, 88.5, 56.4, 55.7, 55.6, 55.1, 55.0, 52.9, 52.0, 51.5. IR (cm^{-1}): 3420, 2980, 1760, 1720, 1620, 1600, 1520, 1480, 1260, 1230, 1180, 1160, 1120, 1100, 1020, 820, 710. HRMS *m/z*: 491.1685 (M + H, Calcd for $C_{28}H_{27}O_8$, 491.1738). **3b**: 0.10 g, yield: 10.5%. mp 196–198°C. ^{1}H NMR($CDCl_3$): δ 3.17 (s, 1H), 3.67 (s, 3H), 3.72 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 4.93 (s, 1H), 5.60 (s, 1H), 6.10 (d, 1H, J = 1.8 Hz), 6.35 (d, 1H, J = 1.8 Hz), 6.65–6.68 (m, 2H), 7.15–7.24 (m, 3H), 7.29–7.35 (m, 4H). ^{13}C NMR: δ 200.8, 170.5, 162.1, 158.9, 156.4, 130.4, 129.2, 129.0, 127.6, 127.4, 127.2, 126.4, 113.8, 101.5, 95.6, 92.9, 77.4, 77.0, 76.5, 75.9, 61.4, 55.8, 55.4, 55.2, 53.0, 52.9, 51.6, 50.9. IR (cm^{-1}): 3429, 1739, 1682, 1604, 1384, 1259, 1201, 1144, 1096, 852, 707. HRMS *m/z*: 491.1742 (M + H, Calcd for $C_{28}H_{27}O_8$, 491.1738).

(2) **3c** and **3d**: Following the same procedure as previously mentioned, starting from **2b** to prepare **3c** and **3d**.

3c: 0.25 g, yield: 26.2%, mp 95–96°C. ^{1}H NMR ($CDCl_3$): δ 2.95 (s, 1H), 3.42 (s, 3H), 3.49 (s, 3H), 3.61 (s, 3H), 3.63 (s, 3H), 4.08 (d, 1H, J = 13.0 Hz), 4.16 (d, 1H, J = 13.0 Hz), 5.30 (d, 1H, J = 1.8 Hz), 5.60 (d, 1H, J = 1.8 Hz), 6.63–6.67 (m, 2H), 6.90–7.01 (m, 4H), 7.08–7.65 (m, 3H). IR (cm^{-1}): 3400, 2980, 1770, 1720, 1600, 1500, 1480, 1430, 1420, 1350, 1260, 1220, 1150, 1120, 820, 710. HRMS *m/z*: 491.1735 (M + H, Calcd for $C_{28}H_{27}O_8$, 491.1738). **3d**, 0.12 g, yield: 12.5%. mp 163–164°C. ^{1}H NMR ($CDCl_3$): δ 3.17(s, 1H), 3.68 (s, 3H), 3.71 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 4.95 (s, 1H), 6.16 (s, 1H), 5.96 (d, 1H, J = 1.8 Hz), 6.24 (d, 1H, J = 1.8 Hz), 6.62–6.67 (m, 2H), 7.15–7.24 (m, 3H), 7.29–7.34 (m, 4H). ^{13}C NMR: δ 200.7, 170.6, 162.1, 158.9, 156.4, 115.1, 130.9, 129.9, 129.2, 127.7, 127.4, 127.2, 126.4, 113.8, 101.5, 95.5, 92.9, 77.4, 77.0, 76.5, 75.9, 61.4, 55.8, 55.4, 55.2, 53.0, 52.9, 51.5, 50.9. IR (cm^{-1}): 3249, 1739, 1681, 1605, 1384, 1259, 1144, 1095, 812, 708. HRMS *m/z*: 491.1729 (M + H, Calcd for $C_{28}H_{27}O_8$, 491.1738).

Synthesis of 4. To a solution of **3a**(0.1 g, 0.20 mmol) in anhydrous methanol (10 mL) was added a solution of $NH_2NH_2.H_2O$ (0.013 g, 0.22 mmol) in methanol (2 mL). The mixture was refluxed for 6 h and left to stand at room temperature overnight and was then filtered to afford colorless solid 0.078 g (82.6%), mp: 280–282°C.^{1}H NMR ($CDCl_3$) δ: 3.49 (s, 1H), 3.66 (s, 3H), 3.81 (s, 3H), 3.84 (s, 3H), 4.94 (s, 1H,), 6.07 (d, 1H, J = 1.9 Hz), 6.31 (d, 1H, J = 1.9 Hz), 6.58–6.61 (m, 2H), 7.03–7.28 (m, 8H,). ^{13}C NMR δ: 164.1, 160.7, 158.8, 157.4, 137.5, 128.7, 127.8, 127.7, 126.8, 126.4, 113.0, 112.8, 107.0, 92.6, 89.3, 83.7, 64.3, 55.7, 55.0, 54.9. IR (cm^{-1}): 3189, 1609, 1514, 1456, 1252, 1150, 1113, 1037, 1019, 811, 764, 700. HRMS *m/z*: 473.17031 (M + H, Calcd for $C_{27}H_{25}N_2O_6$, 473.17071).

Synthesis of 5. To a solution of **3a** (0.1 g, 0.20 mmol) in anhydrous methanol (10 mL) was added a solution of phenyl hydrazine (0.023 g, 0.22 mmol) in methanol (3 mL). The mixture was refluxed for 10 h and left to stand at room temperature overnight and was then filtered to afford red solid 0.07 g (60.3% yield), mp 210–212°C. ^{1}H NMR ($CDCl_3$) δ: 3.66 (s, 3H), 3.70 (s, 3H), 3.75 (s, 3H), 3.86 (s, 3H), 4.00 (d, 1H, J = 12.2 Hz), 4.35 (d, 1H, J = 12.2 Hz), 6.10 (d, 1H, J = 1.9 Hz), 6.34 (d, 1H, J = 1.9 Hz), 6.65–6.68 (m, 2H), 6.94–7.24 (m, 11H,), 7.26–7.34 (m, 2H). ^{13}C NMR: δ 171.8, 164.3, 160.4, 159.1, 157.5, 145.2, 138.0, 136.0, 129.2, 128.1, 127.9, 126.9, 125.7, 119.6, 113.5, 112.3, 101.4, 92.9, 90.2, 86.8, 56.1, 55.7, 55.1, 53.0, 52.4, 51.1. IR (cm^{-1}): 3435, 2937, 1738, 1600, 1516, 1384, 1253, 1151, 1019, 805, 749, 696. HRMS *m/z*: 581.22825 (M + H, Calcd for $C_{34}H_{33}N_2O_7$, 581.22823).

Synthesis of 6. To a solution of **3a** (0.1 g, 0.20 mmol) in anhydrous methanol (10 mL) was added a solution of hydroxylamine hydrochloride (0.017 g, 0.25 mmol) in methanol (2 mL). The mixture was refluxed for 12 h, and then the solvent was evaporated and separated by column chromatography (petroleum/ethyl acetate as eluent) to afford red solid 0.07 g (60.3% yield), mp 203–205°C.

^{1}H NMR ($CDCl_3$) δ: 2.98 (s, 3H), 3.51 (s, 3H), 3.74 (s, 3H), 3.87 (s, 3H), 3.80 (d, 1H, J = 13.2 Hz), 4.20 (d, 1H, J = 13.2 Hz), 6.19 (d, 1H, J = 1.9 Hz), 6.32 (d, 1H, J = 1.9 Hz), 6.67–6.70 (m, 2H), 6.82–7.10 (m, 7H,). ^{13}C NMR δ: 171.0, 164.4, 161.1, 160.0, 158.8, 157.3, 134.9, 128.3, 128.0, 127.8, 127.7, 127.1, 126.2, 113.1, 103.5, 99.0, 93.0, 91.4, 89.4, 71.7, 64.3, 55.9, 55.8, 55.7, 55.6, 52.5, 52.3, 47.2. IR (cm^{-1}): 3205, 2948, 1736, 1613, 1598, 1518, 1384, 1252, 1116, 1046, 974, 838, 814, 700. HRMS *m/z*: 520.19624 (M + H, Calcd for $C_{29}H_{30}NO_8$, 520.19659); 542.17786 (M + Na, Calcd for $C_{29}H_{29}N_2O_8Na$, 542.17854).

BIOASSAY

Azadirachtin and the newly synthesized compounds were individually dissolved in chloroform (1% M/V) and

then diluted with water containing 0.02% emulsifier and 0.05% Triton X-100 to the tested concentrations.

Insect repellency. The leaf of *Brassica chinensis* Linn. was washed, dried by airing, and cut as a Ø70-mm round disk, which was then divided into two halves. One half of the disk was daubed with 0.2 mL tested agent prepared earlier on both sides. The other half of the disk was a control, which was only daubed with water containing emulsifier and Triton X-100. After being dried by airing, the disks were put in an Ø85-mm culture dish and 10 third instar larvae of *P. xylostella* (Linnaeus) were put on the half leaf disk containing insecticide, covered with cling film, and kept at $(27 \pm 1)^\circ$C for 24 h. Experiments were replicated three times at every concentration. The contents of worm at each treated or control diet was counted, and the repellency (%) was calculated by the following formula:

$$\text{Repellency } (\%) = (C - E)/T \times 100\%$$

where C is the insect numbers in the negative control half of the leaf disk, E is the insect numbers in the treated half of disk, and T is the number of total insects. C, E, and T were the mean data of the three replicates.

Insecticidal activity. The third instar larvae of *Pieris rapae* Linnaeus, *Brassica napus* Linn., and second instar larvae of *L. exigua* (Hubner) were treated with Potter's method under 200 and 100 $\mu g\,mL^{-1}$. The leaf of *B. chinensis* Linn. was cut to Ø15-mm round disks. These leaf disks were immersed in sample solution for 10 s, dried by airing, and then put in a testing box with ten holes, which in every hole a piece of leaf disk was needed. The third instar larva of *Helicoverpa armigera* was placed in the hole and covered with cling film. Ten insects were used at every concentration; all experiments were kept at $(27 \pm 1)^\circ$C and were replicated six times. The mortality of insects was determined after 3 days, and Abbotts formula was used to correct the mortality relative to that of negative control. The data was presented in the form of mean mortality (%).

Acknowledgments. We would like to thank the National Natural Science Foundation of China (No. 20772151) and Hebei University Young Foundation (2010Q13) for financial support. We are most grateful to Professor Rui Changhui (Institute of Plant Protection, Chinese Academy of Agriculture Science) for his kind assistance with the bioassay study.

REFERENCES AND NOTES

[1] Li, H. -S.; Fu, B.; Li, N.; Dong, Y.-H.; Qin, Zh. -H. Chin J Org Chem 2005, 25(2), 141.

[2] Nugroho, B. W.; Edrada, R. A.; Wray, V.; Witte, L.; Bringmann, G.; Gehling, M.; Proksch, P. Phytochem 1999, 51, 367.

[3] Greger, H.; Pacher, T.; Brem, B.; Bacher, M.; Hofer, O. Phytochem 2001, 57, 57.

[4] Hiort, C. J.; Nugroho, B. W.; Bohnenstengel, F. I.; Wray, V.; Witte, L.; Hung, P. D.; Kiet, L. C.; Sumaryono, W.; Proksch, P. Phytochem 1999, 52, 837.

[5] Li, H.-S.; Fu, B.; Wang, M. A.; Li, N.; Liu, W. J.; Xie, Z. Q.; Ma, Y. Q.; Qin, Zh.-H. Eur J Org Chem 2008, 1753.

[6] Wang, X. Y.; Li, N.; Ma, Y. Q.; Qin, Zh.-H. Acta Cryst 2009, E65, o2893.

[7] Tsuritani, T.; Ito, S.; Shinokubo, H.; Oshima, K. J Org Chem 2000, 65, 5066.

[8] Askham, F. R.; Carroll, K. M. J Org Chem 1993, 58, 7328.