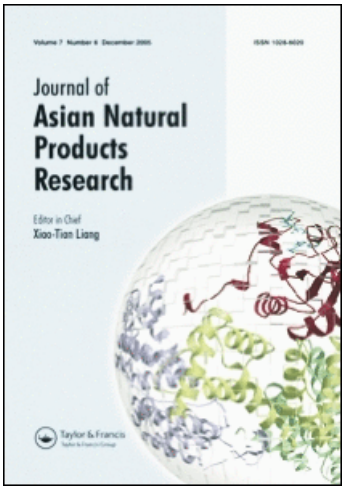

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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[a] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China [b] Faculty of Conservation Biology, Southwest Forestry University, Kunming, China [c] The Life Science and the Technical Institute, Central South University of Forestry and Technology, Changsha, China [d] College of Life and Environmental Sciences, Minzu University of China, Beijing, China [e] Yunnan Agricultural University, Kunming, China [f] Henan Institute of Science and Technology, Xinxiang, China [g] Graduate University of Chinese Academy of Science, Beijing, China

Online publication date: 01 February 2010

To cite this Article Niu, Hong-Mei, Zeng, Dong-Qin, Long, Chun-Lin, Peng, Ying-Hui, Wang, Yue-Hu, Luo, Ji-Feng, Wang, Hong-Sheng, Shi, Ya-Na, Tang, Gui-Hua and Zhao, Fu-Wei(2010) 'Clerodane diterpenoids and prenylated flavonoids from *Dodonaea viscosa*', Journal of Asian Natural Products Research, 12: 1, 7 — 14
To link to this Article: DOI: 10.1080/10286020903407379
URL: http://dx.doi.org/10.1080/10286020903407379

Journal of Asian Natural Products Research
Vol. 12, No. 1, January 2010, 7–14

ORIGINAL ARTICLE

Clerodane diterpenoids and prenylated flavonoids from *Dodonaea viscosa*

Hong-Mei Niu[ab], Dong-Qin Zeng[c], Chun-Lin Long[abd]*, Ying-Hui Peng[c]*, Yue-Hu Wang[a], Ji-Feng Luo[a], Hong-Sheng Wang[aef], Ya-Na Shi[ae], Gui-Hua Tang[a] and Fu-Wei Zhao[ag]

[a]*Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China;* [b]*Faculty of Conservation Biology, Southwest Forestry University, Kunming 650224, China;* [c]*The Life Science and the Technical Institute, Central South University of Forestry and Technology, Changsha 410004, China;* [d]*College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China;* [e]*Yunnan Agricultural University, Kunming 650201, China;* [f]*Henan Institute of Science and Technology, Xinxiang 453003, China;* [g]*Graduate University of Chinese Academy of Science, Beijing 100049, China*

(*Received 17 August 2009; final version received 13 October 2009*)

Repeated column chromatography of the EtOAc-soluble fraction of the aerial parts of *Dodonaea viscosa* led to the isolation of two new modified clerodanes, methyl dodovisate A (**1**) and methyl dodovisate B (**2**), two new prenylated flavonoids, 5,7,4′-trihydroxy-3′,5′-di(3-methylbut-2-enyl)-3,6-dimethoxyflavone (**10**) and 5,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methylbut-2-enyl)-3,6-dimethoxyflavone (**11**), together with eight known compounds, dodonic acid (**3**), hautriwaic acid (**4**), hautriwaic lactone (**5**), (+)-hardwickiic acid (**6**), 5α-hydroxy-1,2-dehydro-5,10-dihydroprintzianic acid methyl ester (**7**), strictic acid (**8**), dodonolide (**9**), and aliarin (**12**). The structures of the new compounds were elucidated by spectroscopic data analysis. Compounds **1**–**9** and **11** were evaluated on larvicidal activity against the fourth-instar larvae of *Aedes albopictus* and *Culex pipens quinquefasciatus*.

Keywords: Sapindaceae; *Dodonaea viscosa*; clerodane diterpenoids; prenylated flavonoids

1. Introduction

Dodonaea viscosa (Linn.) Jacq. (Sapindaceae) is a shrub, rarely a small tree, and widely distributed in tropical and subtropical areas of both hemispheres. It is used in folk medicine as a febrifuge, a diaphoretic drug, and also for the treatment of rheumatism, gout [1], inflammations, swelling, and pain [2]. According to Indian folk medicines, the seeds of *D. viscosa* are used as a fish poison [1]. Recently, the crude extracts of *D. viscosa* have demonstrated contact toxicity against *Sitophilus oryzae* [3], and antifeedant activities on *Plutella xylostella* [4] and *Pieris rapae* [5,6]. Several flavonoids, diterpenoid acids, and saponins have been found from this species [7]. However, the chemical basis for the pesticidal and antifeedant activities is unclear.

In our present research on this plant, a series of clerodane diterpenoids and

*Corresponding authors. Email: long@mail.kib.ac.cn; pengyh999@163.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903407379
http://www.informaworld.com

prenylated flavonoids, including four new ones, are isolated from the aerial parts of *D. viscosa*. Repeated column chromatography of the EtOAc-soluble fraction of the aerial parts of *D. viscosa* led to the isolation of four new (**1**, **2**, **10**, **11**) and eight known compounds (Figure 1). Furthermore, the isolates were evaluated on larvicidal activity against the fourth-instar larvae of *Aedes albopictus* and *Culex pipens quinquefasciatus*. In the present paper, we report the structural elucidation of the new compounds and the results of the bioassay.

2. Results and discussion

Compound **1** was obtained as a yellow oil. Its molecular formula was determined as $C_{21}H_{26}O_3$ from its HR-ESI-MS at *m/z* 349.1771 $[M+Na]^+$. The IR spectrum of **1** indicated the presence of a carbonyl group (1707 cm^{-1}). The ^1H NMR spectral data in pyridine-d_5 for **1** exhibited one methoxy signal at δ 3.68 (3H, s, OCH_3-18), two methyl signals at δ 0.77 (3H, d, $J = 5.9$ Hz, H-17) and 0.87 (3H, s, H-20), and protons for the furan ring at δ 6.43 (1H, s, H-14), 7.59 (1H, s, H-15), and 7.47 (1H, s, H-16). The ^{13}C NMR spectral data showed 21 carbon signals including a signal for the methoxy group at δ 51.8 (OCH_3-18), which implied that **1** might be a diterpene.

The planar structure of **1** was elucidated by extensive analyses of its 2D NMR spectra. The $^1H-^1H$ COSY experiment established the connectivity from C-1 to C-3 (fragment **a**, Figure 2), C-6 to C-8 and then to C-17 (fragment **b**), and C-11 to C-12 (fragment **c**). Based on the presence of fragments **a** and **b** and the HMBC correlations (Figure 2) of H-1/C-5 and C-9, H-2/C-4 and C-10, H-3/C-19, H-6/C-10 and C-19, H-7/C-5 and C-9, H_3-17/C-9, and H_3-20/C-10, the 7/6 dicyclic core of the diterpene was established. The furan ring was located at C-12 of fragment **c** and then the fragment at C-9 by the HMBC correlations of H-11/C-8, C-10, and C-13, and H-12/C-14. The ester carbonyl was attached to C-4 on the basis of the HMBC correlation from H-3 to C-18, and the methoxy group to C-18 by the correlation from OCH_3-18 to C-18. Thus, **1** was deduced as a rare 5(4 → 19)-*abeo*-clerodane derivative.

The relative stereochemistry was deduced from the ROESY spectrum of **1**. The crucial ROESY correlation (Figure 2) between H-8 and H_2-11 suggested that these protons were cofacial. H-8 was arbitrarily assigned as being in a β-orientation, while H_3-17 and H_3-20 were in an α-orientation. Therefore, compound **1** was elucidated as 5(4 → 19)-*abeo*-15,16-epoxy-1,3,5(10),13(16),14-clerodapentaen-18-oic acid methyl ester, named methyl dodovisate A. Because the chemical shifts of H_3-17 and H_3-20 are very closely measured in $CDCl_3$ (see Section 3), those measured in pyridine-d_5 were employed to discuss the relative configuration of **1**.

Compound **2** was obtained as a yellow oil. Its molecular formula was determined to be $C_{21}H_{26}O_4$ by HR-ESI-MS at *m/z* 365.1737 $[M+Na]^+$. The IR spectrum of **2** indicated a carbonyl group (1746 cm^{-1}). Careful comparison of the ^1H and ^{13}C NMR spectral data of **2** with those of **1** implied that compound **2** was very similar to **1** except for the absence of the typically substituted furan signals and the appearance of the characteristic signals for a butenolide moiety [δ_H 7.14 (1H, m, H-14) and 4.72 (2H, br s, H-15); δ_C 134.4 (C-13), 145.3 (C-14), 70.6 (C-15), and 175.0 (C-16)] in **2**. The butenolide moiety was attached to C-12 by the HMBC correlations of H-12/C-14. The crucial ROESY correlation of H_2-11/H-8 revealed that the relative configuration of **2** was the same as that of **1**. Therefore, the structure of **2** was elucidated as 5(4 → 19)-*abeo*-1,3,5(10),13-clerodatetraen-16,15-olid-18-oic acid methyl ester, named methyl dodovisate B.

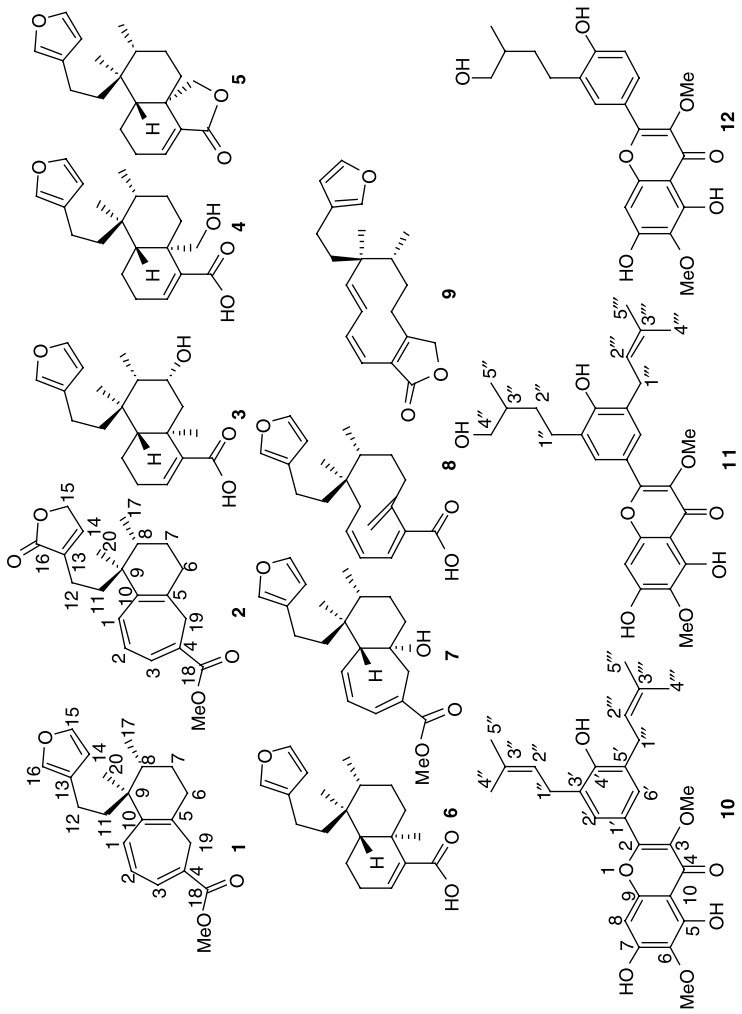

Figure 1. Structures of compounds **1**–**12**.

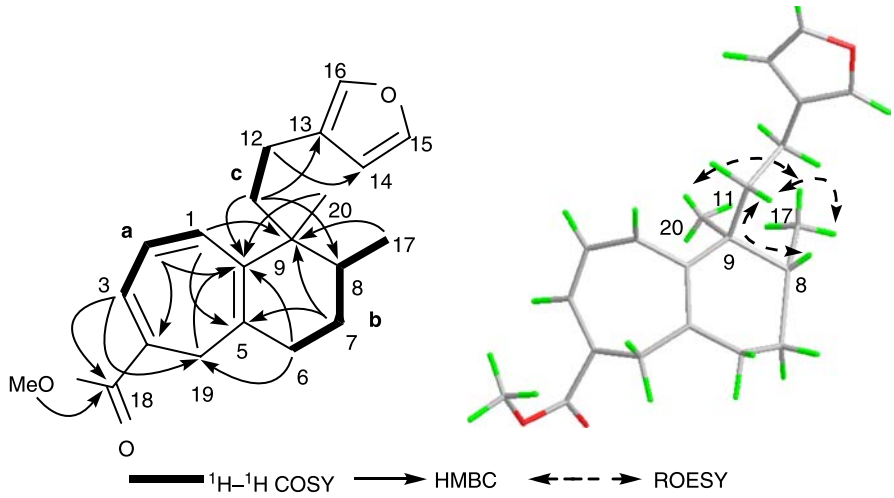

Figure 2. Key 2D NMR correlations of **1**.

Compound **10**, a yellow amorphous powder, was assigned the molecular formula $C_{27}H_{30}O_7$ by HR-ESI-MS at *m/z* 489.1882 $[M+Na]^+$. The IR spectrum of **10** indicated the presence of hydroxy groups (3421 cm^{-1}), a carbonyl group (1654 cm^{-1}), and aromatic groups (1611 and 1559 cm^{-1}). The UV absorption bands (241, 272, and 341 nm) and ^{13}C NMR signal at δ_C 179.2 (C-4) were characteristic of a flavone nucleus. The NMR spectra showed two methoxy signals at δ 3.76 (3H, s, OCH_3-3) and 3.96 (3H, s, OCH_3-6) and two 3-methylbut-2-enyl groups [δ_H 1.74 (12H, s, H-4″, H-4‴, H-5″, and H-5‴), 3.35 (4H, d, $J = 7.1$ Hz, H-1″ and H-1‴), 5.28 (2H, t, $J = 7.1$ Hz, H-2″ and H-2‴); δ_C 17.9 (C-4″ and C-4‴), 25.8 (C-5″ and C-5‴), 29.7 (C-1″ and C-1‴), 121.3 (C-2″ and C-2‴), 135.3 (C-3″ and C-3‴)]. The two prenyl groups were located at C-3′ and C-5′ by the HMBC correlations from H-2″ and H-2‴ to C-3′ and C-5′. The substitute patterns of the hydroxy and methoxy groups of **10** were similar to those of known aliarin (**12**) and were confirmed by the HMBC spectrum of **10**. On the basis of these findings, compound **10** was determined to be 5,7,4′-trihydroxy-3′,5′-di(3-methylbut-2-enyl)-3,6-dimethoxyflavone.

Compound **11**, a yellow amorphous powder, was shown to have the molecular formula $C_{27}H_{32}O_8$ by HR-ESI-MS at *m/z* 507.1987 $[M+Na]^+$. Careful comparison of the ^1H and ^{13}C NMR spectral data of **11** with those of known aliarin (**12**) implied that compound **11** was very similar to aliarin except for the appearance of the signals for a 3-methylbut-2-enyl group [δ_H 3.31 (2H, m, H-1‴), 5.31 (1H, t, $J = 7.3$ Hz, H-2‴), 1.68 (3H, s, H-4‴), 1.72 (3H, s, H-5‴); δ_C 28.3 (C-1‴), 122.2 (C-2‴), 132.5 (C-3‴), 17.7 (C-4‴), 25.6 (C-5‴)] in the NMR spectra of **11**. This group was located at C-5′ by the HMBC correlations from H-2‴/C-5′ and H_2-1‴/C-4′. The assignments of C-4‴ and C-5‴ were obtained from the correlations of H_2-1‴/H_3-4‴ and H-2‴/H_3-5‴ in the ROESY spectrum of **11**. Thus, compound **11** was determined to be 5,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methylbut-2-enyl)-3,6-dimethoxyflavone.

The structures of the known compounds, dodonic acid (**3**) [8,9], hautriwaic acid (**4**) [8,9], hautriwaic lactone (**5**) [9,10], (+)-hardwickiic acid (**6**) [11], 5α-hydroxy-1,2-dehydro-5,10-dihydroprintzianic acid methyl ester (**7**) [12], strictic acid (**8**) [13,14], dodonolide (**9**) [15], and

aliarin (**12**) [16], were identified by comparing their spectroscopic data with those of the published values.

The toxicities of compounds **1**–**9** and **11** were evaluated on the larvae of *C. pipens quinquefasciatus* and *A. albopictus*. However, all of them were inactive ($LC_{50} > 30\ \mu g/ml$). Clerodane diterpenoids have been reported to possess insect antifeedant activity [17,18]. It is worthwhile to further evaluate the antifeedant activity of these isolates from *D. viscosa*.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were determined on a Shimadzu double-beam 210A spectrometer. IR spectra were measured on a Bio-Rad FTS-135 infrared spectrophotometer with KBr disks. 1D (^1H and ^{13}C NMR) and 2D NMR (^1H–^1H COSY, HMQC, HMBC, and ROESY) spectra were obtained on BRUKER AM-400 and DRX-500 spectrometers with TMS as the internal standard. MS analyses were performed on a VG Auto Spec-3000 mass spectrometer. Semipreparative HPLC was carried out on an Agilent 1200 series pump equipped with a diode array detector and a Zorbax SB-C18 column (5.0 μm, ϕ 9.4 × 250 mm). Silica gel G (80–100 and 300–400 mesh; Qingdao Makall Group Co. Ltd, Qingdao, China), RP_{18} silica gel (40–75 μm; Fuji Silysia Chemical Ltd, Tokyo, Japan), silica gel H (10–40 μm; Qingdao Makall Group Co. Ltd), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography, and silica gel GF_{254} (Qingdao Makall Group Co. Ltd) for preparative TLC as precoated plates. The TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in alcohol, followed by heating.

3.2 Plant material

The aerial parts (branches, leaves, and fruits) of *D. viscosa* were obtained from Mile County, Yunnan Province, China, in November 2007, and identified by Prof. Chun-Lin Long at Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (ML0701) is deposited at the Research Group for Biodiversity and Plant Resources, Kunming Institute of Botany, the Chinese Academy of Sciences.

3.3 Extraction and isolation

The milled aerial parts of *D. viscosa* (4 kg) were extracted three times with MeOH under reflux. The methanolic extracts (665 g) were dissolved in H_2O and partitioned successively with petroleum ether and EtOAc. The EtOAc extract (348 g) was subjected to chromatography over a silica gel column ($CHCl_3$–MeOH, 1:0 → 1:1) to yield fractions A–E. Fraction A (82 g) was chromatographed over an RP_{18} silica gel column (MeOH–H_2O, 85:25 → 1:0) to give subfractions A_1–A_9. Fraction A_7 (3 g) was subjected to a silica gel column (petroleum ether–EtOAc, 30:1 → 0:1) to give subfractions A_{71}–A_{75}. Fraction A_{72} (338 mg) was subjected to chromatography over silica gel (petroleum ether–EtOAc, 15:1) and preparative TLC ($CHCl_3$–MeOH, 30:1) to give compound **6** (14.6 mg). Fraction A_{74} (796 mg) was chromatographed over a silica gel column (petroleum ether–EtOAc, 5:1) and then purified by preparative TLC ($CHCl_3$–MeOH, 100:1) to afford compounds **9** (13.2 mg), **7** (6.0 mg), and **8** (16.0 mg), and another fraction. The latter was subjected to semipreparative HPLC (H_2O–CH_3CN, 20:80) to attain compound **2** (11.6 mg). Fraction A_8 (532 mg) was fractionated on a Sephadex LH-20 column (MeOH), silica gel column (petroleum ether–EtOAc, 2:1), and then on preparative TLC ($CHCl_3$–MeOH, 80:1) to afford compounds **1** (34.7 mg), **5** (12.1 mg), and **10** (10.4 mg). Fraction B (64 g) was

chromatographed over an RP_{18} silica gel column (MeOH–H_2O, 75:25 → 1:0) to give subfractions B_1–B_{14}. Fraction B_5 (4 g) was chromatographed over a Sephadex LH-20 column (MeOH) and silica gel chromatography (petroleum ether–EtOAc, 2:1) to afford compound **12** (72.6 mg). Fraction B_9 (2 g) was chromatographed over a Sephadex LH-20 column (MeOH) to yield compounds **3** (38.6 mg) and **4** (37.3 mg), and another fraction. The latter was subjected to silica gel column (petroleum ether–EtOAc, 2:1), preparative TLC (EtOAc; $CHCl_3$–MeOH, 30:1), and Sephadex LH-20 (MeOH) to obtain compound **11** (36.0 mg).

3.3.1 *Methyl dodovisate A (**1**)*

A yellow oil: $[\alpha]_D^{18}$ +56.4 ($c = 0.26$, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) nm: 324 (3.41), 271 (3.53), 239 (3.60), 210 (3.40), 206 (3.40); IR (KBr) ν_{max}: 3447, 1707, 1502, 1436, 1280, 1215, 1086, 873, 760, 599 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N): δ 7.04 (1H, d, $J = 11.2$ Hz, H-1), 6.63 (1H, dd, $J = 11.7$, 5.6 Hz, H-2), 7.19 (1H, d, $J = 5.6$ Hz, H-3), 2.44 (1H, m, H-6), 2.32 (1H, m, H-6), 1.40 (1H, m, H-7), 1.38 (1H, m, H-7), 1.69 (1H, m, H-8), 1.82 (2H, m, H-11), 2.32 (1H, m, H-12), 2.04 (1H, m, H-12), 6.43 (1H, s, H-14), 7.59 (1H, s, H-15), 7.47 (1H, s, H-16), 0.77 (3H, d, $J = 5.9$ Hz, H-17), 2.84 (1H, m, H-19), 1.69 (1H, m, H-19), 0.87 (3H, s, H-20), 3.68 (3H, s, OCH_3-18); ^{13}C NMR (100 MHz, C_5D_5N): δ 136.5 (C-1), 128.2 (C-2), 132.2 (C-3), 123.6 (C-4), 134.5 (C-5), 32.0 (C-6), 27.2 (C-7), 33.4 (C-8), 40.6 (C-9), 135.8 (C-10), 38.6 (C-11), 19.9 (C-12), 126.2 (C-13), 111.7 (C-14), 143.3 (C-15), 139.2 (C-16), 16.0 (C-17), 166.8 (C-18), 33.7 (C-19), 23.4 (C-20), 51.8 (OCH_3-18); ^1H NMR (500 MHz, $CDCl_3$): δ 6.95 (1H, d, $J = 11.7$ Hz, H-1), 6.57 (1H, dd, $J = 11.7$, 5.4 Hz, H-2), 7.08 (1H, d, $J = 5.4$ Hz, H-3), 2.43 (1H, m, H-6), 2.31 (1H, m, H-6), 1.53 (1H, m, H-7), 1.43 (1H, m, H-7), 1.77 (1H, m, H-8), 1.78 (2H, m, H-11), 2.26 (1H, m, H-12), 1.96 (1H, m, H-12), 6.23 (1H, s, H-14), 7.33 (1H, s, H-15), 7.17 (1H, s, H-16), 0.89 (3H, d, $J = 6.4$ Hz, H-17), 2.31 (1H, m, H-19), 1.76 (1H, m, H-19), 0.91 (3H, s, H-20), 3.80 (3H, s, OCH_3-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.7 (C-1), 127.6 (C-2), 132.0 (C-3), 123.6 (C-4), 134.4 (C-5), 31.7 (C-6), 26.9 (C-7), 33.2 (C-8), 40.3 (C-9), 135.9 (C-10), 38.3 (C-11), 19.5 (C-12), 125.6 (C-13), 111.0 (C-14), 142.6 (C-15), 138.4 (C-16), 16.0 (C-17), 166.9 (C-18), 33.3 (C-19), 23.3 (C-20), 51.9 (OCH_3-18); EI-MS *m/z* (%): 326 (20) $[M]^+$, 231 (100), 199 (23), 175 (22), 163 (36), 162 (49), 157 (25), 149 (27), 143 (24), 129 (22); HR-ESI-MS: *m/z* 349.1771 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_3Na$, 349.1779).

3.3.2 *Methyl dodovisate B (**2**)*

A yellow oil: $[\alpha]_D^{18}$ +28.6 ($c = 0.59$, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) nm: 321 (3.11), 269 (3.27), 239 (3.41), 229 (3.22), 222 (3.21), 211 (3.22), 205 (3.22), 196 (3.23), 192 (3.23); IR (KBr) ν_{max}: 3431, 1746, 1698, 1611, 1436, 1350, 1277, 1213, 1073, 761 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N): δ 7.05 (1H, d, $J = 11.5$ Hz, H-1), 6.62 (1H, dd, $J = 11.5$, 5.5 Hz, H-2), 7.19 (1H, m, H-3), 2.43 (1H, m, H-6), 2.36 (1H, m, H-6), 1.38 (1H, m, H-7), 1.31 (1H, m, H-7), 1.76 (1H, m, H-8), 1.83 (2H, m, H-11), 2.22 (1H, m, H-12), 1.98 (1H, m, H-12), 7.14 (1H, m, H-14), 4.72 (2H, br s, H-15), 0.81 (3H, d, $J = 6.6$ Hz, H-17), 2.90 (1H, m, H-19), 2.36 (1H, m, H-19), 0.86 (3H, s, H-20), 3.68 (3H, s, OCH_3-18); ^{13}C NMR (100 MHz, C_5D_5N): δ 136.9 (C-1), 128.3 (C-2), 132.2 (C-3), 123.1 (C-4), 134.2 (C-5), 31.8 (C-6), 27.1 (C-7), 33.7 (C-8), 40.5 (C-9), 135.1 (C-10), 35.7 (C-11), 20.7 (C-12), 134.4 (C-13), 145.3 (C-14), 70.6 (C-15), 175.0 (C-16, disappeared in C_5D_5N and determined by HMBC), 16.0 (C-17), 166.8 (C-18), 33.5 (C-19), 23.6

(C-20), 51.8 (OCH_3-18); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (1H, d, $J = 11.5$ Hz, H-1), 6.59 (1H, dd, $J = 11.5$, 5.6 Hz, H-2), 7.11 (1H, m, H-3), 2.45 (1H, m, H-6), 2.33 (1H, m, H-6), 1.56 (1H, m, H-7), 1.44 (1H, m, H-7), 1.78 (1H, m, H-8), 1.82 (2H, m, H-11), 2.20 (1H, m, H-12), 1.93 (1H, m, H-12), 7.10 (1H, m, H-14), 4.78 (2H, m, H-15), 0.92 (3H, d, $J = 6.8$ Hz, H-17), 2.77 (1H, m, H-19), 2.27 (1H, m, H-19), 0.93 (3H, s, H-20), 3.80 (3H, s, OCH_3-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.3 (C-1), 127.9 (C-2), 131.9 (C-3), 123.7 (C-4), 134.9 (C-5), 31.6 (C-6), 26.9 (C-7), 33.2 (C-8), 40.2 (C-9), 135.4 (C-10), 35.3 (C-11), 20.4 (C-12), 134.6 (C-13), 143.6 (C-14), 70.2 (C-15), 174.3 (C-16), 15.9 (C-17), 166.9 (C-18), 33.2 (C-19), 23.4 (C-20), 51.9 (OCH_3-18); EI-MS *m/z* (%): 342 (3) $[M]^+$, 310 (72), 283 (21), 282 (77), 231 (24), 215 (21), 199 (50), 185 (26), 171 (100), 162 (60), 157 (45), 155 (29), 143 (41), 141 (35), 131 (24), 128 (35), 115 (27), 91 (30); HR-ESI-MS: *m/z* 365.1737 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_4Na$, 365.1728).

*3.3.3 5,7,4′-Trihydroxy-3′,5′-di(3-methylbut-2-enyl)-3,6-dimethoxyflavone (**10**)*

A yellow amorphous powder: UV ($CHCl_3$) λ_{max} (log ε) nm: 341 (3.90), 272 (3.79), 241 (3.81), 217 (3.76), 203 (3.77); IR (KBr) ν_{max}: 3421, 2927, 1654, 1611, 1559, 1468, 1170, 1051, 806 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.90 (OH-5), 5.78 (OH-4′), 6.47 (1H, s, H-8), 3.76 (3H, s, OCH_3-3), 3.96 (3H, s, OCH_3-6), 7.68 (2H, s, H-2′ and H-6′), 3.35 (4H, d, $J = 7.1$ Hz, H-1″ and H-1‴), 5.28 (2H, t, $J = 7.1$ Hz, H-2″ and H-2‴), 1.74 (12H, s, H-4″, H-4‴, H-5″ and H-5‴); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.7 (C-2), 138.2 (C-3), 179.2 (C-4), 151.8 (C-5), 129.9 (C-6), 154.8 (C-7), 93.0 (C-8), 152.5 (C-9), 106.1 (C-10), 60.0 (OCH_3-3), 60.9 (OCH_3-6), 122.2 (C-1′), 128.3 (C-2′ and C-6′), 127.3 (C-3′ and C-5′), 155.5 (C-4′), 29.7 (C-1″ and C-1‴), 121.3 (C-2″ and C-2‴), 135.3 (C-3″ and C-3‴), 17.9 (C-4″ and C-4‴), 25.8 (C-5″ and C-5‴); EI-MS *m/z* (%): 466 (100) $[M]^+$, 451 (32), 449 (18), 423 (15), 397 (23), 367 (15), 337 (19), 283 (4), 69 (36); HR-ESI-MS: *m/z* 489.1882 $[M + Na]^+$ (calcd for $C_{27}H_{30}O_7Na$, 489.1889).

*3.3.4 5,7,4′-Trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methylbut-2-enyl)-3,6-dimethoxyflavone (**11**)*

A yellow amorphous powder: $[\alpha]_D^{18}$ 0 ($c = 0.12$, MeOH); UV (MeOH) λ_{max} (log ε) nm: 337 (3.14), 291 (3.22), 205 (3.56); IR (KBr) ν_{max}: 3456, 2926, 1640, 1551, 1461, 668 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ 12.76 (OH-5), 6.52 (1H, s, H-8), 3.74 (3H, s, OCH_3-3), 3.73 (3H, s, OCH_3-6), 7.64 (2H, br s, H-2′ and H-6′), 2.70 (1H, m, H-1″), 2.60 (1H, m, H-1″), 1.66 (1H, m, H-2″), 1.29 (1H, m, H-2″), 1.54 (1H, m, H-3″), 3.30 (1H, m, H-4″), 3.24 (1H, dd, $J = 10.4$, 6.1 Hz, H-4″), 0.90 (3H, d, $J = 6.1$ Hz, H-5″), 3.31 (2H, m, H-1‴), 5.31 (1H, t, $J = 7.3$ Hz, H-2‴), 1.68 (3H, s, H-4‴), 1.72 (3H, s, H-5‴); ^{13}C NMR (125 MHz, CD_3OD): δ 156.2 (C-2), 137.3 (C-3), 178.2 (C-4), 152.4 (C-5), 131.3 (C-6), 157.8 (C-7), 94.1 (C-8), 151.6 (C-9), 104.5 (C-10), 59.6 (OCH_3-3), 60.0 (OCH_3-6), 120.9 (C-1′), 127.5 (C-2′), 129.7 (C-3′), 155.4 (C-4′), 128.4 (C-5′), 127.2 (C-6′), 27.4 (C-1″), 33.3 (C-2″), 35.1 (C-3″), 66.3 (C-4″), 16.9 (C-5″), 28.3 (C-1‴), 122.2 (C-2‴), 132.5 (C-3‴), 17.7 (C-4‴), 25.6 (C-5‴); EI-MS: *m/z* 507 $[M + Na]^+$; HR-ESI-MS: *m/z* 507.1987 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_8Na$, 507.1994).

3.4 Larvicidal bioassay

Larvae of *A. albopictus* and *C. pipens quinquefasciatus* were reared in a laboratory at 26 ± 2°C with a photoperiod of 14 h light and 10 h dark and 75 ± 5% relative humidity. Wheat flour, yeast powder, and

chicken liver powder in the ratio of 2:1:0.1 were used as the food source. The method of Momin and Nair [19] was employed to conduct the mosquito larvicidal activity test.

Acknowledgements

This work was funded by the grants of the Ministry of Education of China through its 111 and 985 projects (B08044 and MUC 985-3-3), the Ministry of Science and Technology of China (2008FY110400-2-2 and 2005DKA 21006), and the Knowledge Innovation Program of the Chinese Academy of Sciences.

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