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Plant-derived juvenile hormone III analogues and other sesquiterpenes from the stem bark of *Cananga latifolia*

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ABSTRACT

Juvenile hormone III (JH III) is a larval metamorphosis-regulating hormone present in most insect species. JH III was first isolated from the plant, *Cyperus iria* L., but the presence of JH III has not been reported in other plant species. In the present study, proof of the existence of JH III and its analogues from *Cananga latifolia* was established. From an aqueous MeOH extract of *C. latifolia* stem bark, six compounds were isolated along with nine known compounds. These were identified by using spectroscopic analyses as: (2*E*,6*E*,10*R*)-11-butoxy-10-hydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester, (2*E*,6*E*)-3,7,11-trimethyl-10-oxododeca-2,6-dienoic acid methyl ester, (2*E*)-3-methyl-5-[(1*S*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl]-pent-2-enoic acid methyl ester, 1β-hydroxy-3-oxo-4β, 5α,7α-H-eudesmane 11-*O*-α-L-rhamnopyranoside, 4-*epi*-aubergenone 11-*O*-2′,3′-di-*O*-acetyl-α-L-rhamnopyranoside and 4-*epi*-aubergenone 11-*O*-2′,4′-di-*O*-acetyl-α-L-rhamnopyranoside. Three of the previously known compounds, (2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoic acid methyl ester, (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid and (2*E*,6*S*)-3-methyl-6-hydroxy-6-[(2*R*,5*R*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl]-hex-2-enoaic acid methyl ester have now been found in a plant species. Ultra performance liquid chromatography–quadruple time-of-flight mass spectroscopy (UPLC-QTOF/MS) analysis of the chemical constituents of *C. latifolia* showed that several were predominant in the sub-fractions of a *C. latifolia* stem bark extract.

1. Introduction

Juvenile hormones (JHs) are structurally linear sesquiterpenes that are generally synthesized via the mevalonate pathway and may be found in most insect species (Dewick, 2009). Because JHs play critical roles in physiological processes, such as metamorphosis and reproduction, they are being spotlighted as naturally occurring insecticides that are safer and more eco-friendly to plants and humans than synthetic chemical ones (Goodman et al., 2012; Rajashekar et al., 2012; Riddiford, 2012). Of the JHs, JH III (methyl 10*R*,11-epoxy-3,7,11-trimethyl-2*E*,6*E*-dodecadienoate) is, to date, the first insect hormone isolated from a plant, *Cyperus iria* L. (Toong et al., 1988). The insecticidal activity of JH III analogues may form part of a defensive strategy of plants against insect herbivores by preventing the development from insect larvae to insect adults (Chaitanya et al., 2012).

Cananga latifolia (Hook.f. & Thomson) Finet & Gagnep (Annonaceae) is a deciduous tree native to South-East Asian countries, such as in Cambodia, Thailand, Laos and Vietnam (Saralamp, 1996). In Cambodian traditional medicine, the bark of *C. latifolia* has been used for the treatment of dizziness and fever (Gyatso, 2000). Another member of the *Cananga* genus, *C. odorata*, has been used in perfumery because of the sweet scent of its flower and in aromatherapy because of its action in relief of stress and depression (Hongratanaworakit and Buchbauer, 2004, 2006). Some constituents including alkaloids, monoterpenes and sesquiterpenes have been reported as being present in *C. odorata* (Hsieh et al., 2001; Leboeuf et al., 1975; Matsunami et al., 2010; Nagashima et al., 2010). However, the constituents of *C. latifolia* have not been intensively studied, except for a study on the isolation of linear acetogenins from its stem bark (Wongsa et al., 2011). In this study, isolation of six sesquiterpenes (**1**–**6**), and nine other known compounds (**7**–**15**), from the stem bark of *C. latifolia* is reported, together with JH III and its analogues being abundant in *C. latifolia* extracts.

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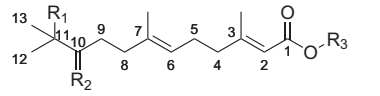
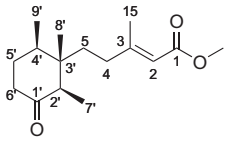
2. Results and discussion

The 80% methanolic extract of *C. latifolia* was successively fractionated with CH_2Cl_2, *n*-BuOH and H_2O. The CH_2Cl_2 and *n*-BuOH fractions were subjected to repeated column chromatographic steps to yield six compounds (**1**–**6**) and nine known compounds (**7**–**15**), whose structures were elucidated by using 1D and 2D NMR spectroscopic, and MS data (Fig. 1). Nine compounds (**7**–**15**) were identified as: JH III (**7**) (Rodriguez and Gros, 1990; Toong et al., 1988); (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester (**8**) (Jacobs et al., 1987); (2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoic acid methyl ester (**9**) (Mori et al., 1975); (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid (**10**) (Findlay et al., 2003); (2*E*,6*S*)-3-methyl-6-hydroxy-6-[(2*R*,5*R*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl]-hex-2-enoaic acid methyl ester (**11**) (Hioki et al., 2009; Messeguer et al., 1991); (1*S*,2*R*,5*S*)-2, 5-dihydroxymethyl-2-hydroxyethyl-cyclohexanol (**12**) (Nagashima et al., 2010); (9*S*,10*E*,12*Z*,15*Z*)-9-hydroxyoctadeca-10,12,15-trienoic acid (**13**) (McLean et al., 1996; Shimura et al., 1983); (9*Z*,11*E*,13*S*,15*Z*)-13-hydroxyoctadeca-9,11,15-trienoic acid (**14**) (Shimura et al., 1983; Waridel et al., 2004) and kalasinamide (**15**) (Gandy and Piggott, 2008) by comparing the spectroscopic data in the literature.

Compound **1** was obtained as a colorless syrup and its molecular formula was concluded to be $C_{20}H_{36}O_4$ by a quasi-molecular ion peak at *m*/*z* 341.2690 $[M+H]^+$ (calcd. for 341.2692) from a positive mode high-resolution FABMS. The ^1H and ^{13}C NMR spectra of **1** were similar to those of methyl 10*R*,11-dihydroxy-3,7,11-trimethyl-2*E*,6*E*-dodecadienoate (**8**) with the exception of the signals at δ_H/δ_C 3.32 (1H, dd, *J* = 2.2, 6.5 Hz, H-1′)/60.7 (C-1′), 1.46 (2H, m, H-2′)/32.6 (C-2′), 1.33 (1H, m, H-3′)/19.4 (C-3′) and 0.89 (3H, t, *J* = 7.4 Hz, H-4′)/13.9 (C-4′). The substituent was deduced to be an *n*-butyl chain and its attachment location was determined to be an oxygen moiety at C-11 on the basis of the correlation peak from δ_H 3.32 (H-1′) to δ_C 76.6 (C-11) (Fig. 2). With the exception of the



	R_1	R_2	R_3
1	*O*-$CH_2CH_2CH_2CH_3$	α-H, β-OH	CH_3
2	H	*O*	H
8	OH	α-H, β-OH	CH_3
10	OH	α-H, β-OH	H



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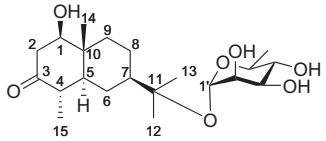

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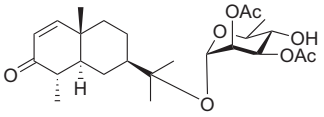

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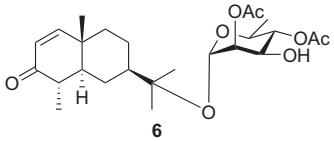

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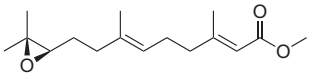

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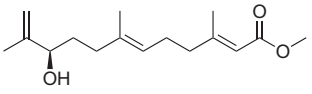

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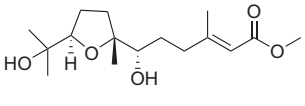

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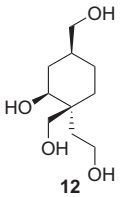

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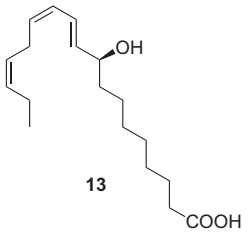

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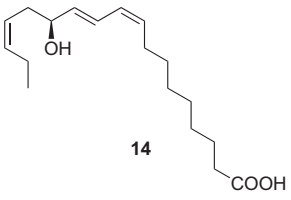

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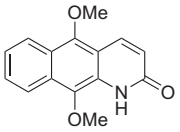

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Fig. 1. The structures of compounds **1**–**15**.

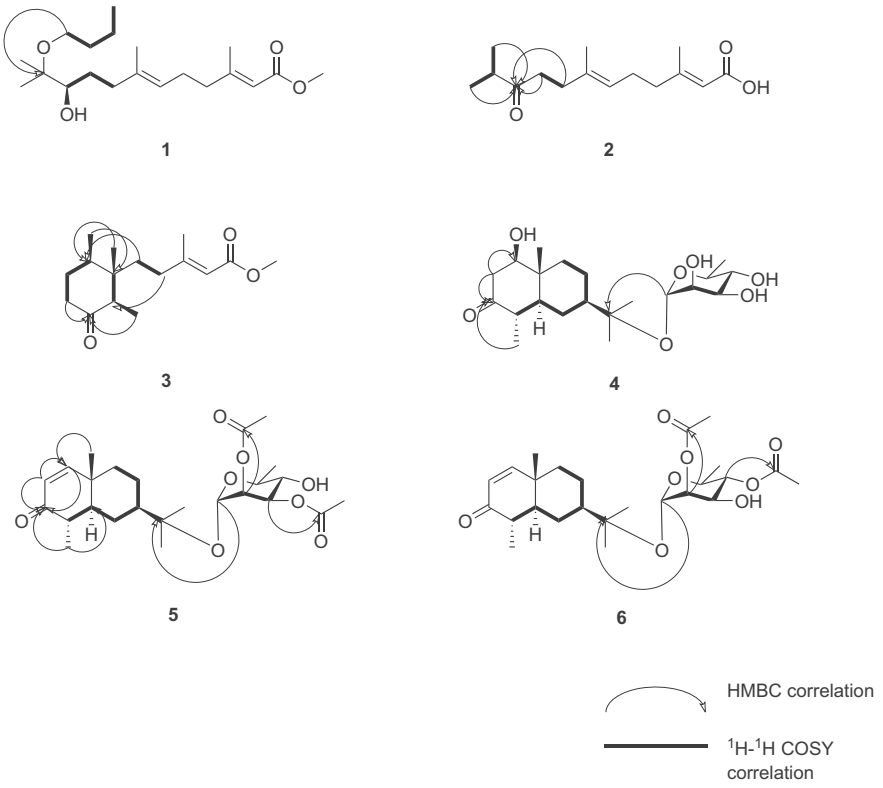

Fig. 2. The key correlations of compounds **1–6**.

butyl substituent, the spectroscopic signals of **1** were identical to those of **8**. The optical rotation value of **1** was $[\alpha]^{20}_D$ +16.7, a value that is consistent with the value ($[\alpha]^{20}_D$ +18.9) of **8** (Nkunya et al., 2000). On the basis of this result, the absolute configuration of the hydroxyl moiety at C-10 was assigned as *R*. Butylated compounds are rare in natural products and can be produced through extraction, fractionation, and isolation (Deng et al., 2008; Liang et al., 2008). However, compound **1** was obtained from the EtOAc fraction without the use of *n*-BuOH. Moreover, compound **1** was not produced by the reaction of **8** with *n*-BuOH under normal experimental conditions. Consequently, the structure of **1**, as a natural product derived from *C. latifolia*, was named as (2*E*,6*E*,10*R*)-11-butoxy-10-hydroxy-3,7,11-trimethyldodeca-2,6-dienoic acid methyl ester.

Compound **2** showed a quasi-molecular ion peak at *m*/*z* 253.1791 $[M+H]^+$ (calcd. for 253.1798) in its positive mode high resolution ESI-MS, consistent with a molecular formula of $C_{15}H_{25}O_3$. The ^{1}H, ^{13}C and HSQC NMR spectral data of **2** clearly showed a ketone carbon at δ_C 214.5 (C-10), a carboxylic group at δ_C 171.5 (C-1), two olefinic protons at δ_H/δ_C 5.65 (1H, s, H-2)/115.1 (C-2) and 5.06 (1H, m, H-6)/123.2 (C-6), and four methyl groups at δ_H 2.16 (3H, s, H-15)/19.1 (C-15), 1.60 (6H, d, *J* = 7.0 Hz, H-12 and H-13)/18.2 (C-12 and C-13) and 1.58 (3H, s, H-14)/16.1 (C-14). On the basis of a ^{1}H–^{1}H COSY spectoscopic experiment, the correlations from H-11 (1H, m, δ_H 2.58) to H-12 and H-13 and from H-8 (1H, t, *J* = 7.8 Hz, δ_H 2.21) to H-9 (1H, t-like, *J* = 7.8 Hz, δ_H 2.51) indicated the linkage of H-11/(H-12 and H-13) and H-8/H-9. In the HMBC spectrum, the signals at δ_H 1.06 (H-12 and H-13), 2.51 (H-9) and 2.21 (H-8) had cross-peaks with δ_C 214.5 (C-10) (Fig. 2). These results suggested that an isopropyl ketone was present in **2**. The remaining spectroscopic data were consistent with the data of a 3,7-dimethyl-2*E*, 6*E*-heptadienoate in the structure of **10**. On the basis of these data, **2** was assigned as (2*E*,6*E*)-3,7,11-trimethyl-10-oxododeca-2,6-dienoic acid methyl ester.

Compound **3** was isolated as a yellow syrup and high resolution ESI-MS (positive ion mode, *m*/*z* 267.1948 $[M+H]^+$ (calcd. for 267.1955)) data indicated that the molecular formula of **3** was $C_{16}H_{26}O_3$. In the 1D NMR, HMQC and DEPT spectra, the structure of **3** consisted of five methyl groups (δ_H/δ_C 3.67 (3H, s, OC<u>H</u>$_3$)/50.9 (O<u>C</u>H$_3$), 2.17 (3H, s, H-6)/19.1 (C-6), 0.91 (3H, d, *J* = 8.4 Hz, H-7′)/7.6 (C-7′), 0.88 (3H, d, *J* = 8.5 Hz, H-9′)/15.0 (C-9′) and 0.58 (3H, s, H-8′)/15.3 (C-8′), four methylene groups (δ_H/δ_C 2.34 (2H, m, H-6′)/41.5 (C-6′), 2.14 and 1.99 (2H, m, H-4)/34.1 (C-4), 1.83 and 1.62 (2H, m, H-5′)/30.8 (C-5′), and 1.44 (2H, m, H-5)/35.2 (C-5)) three methyl groups (δ_H/δ_C 5.67 (1H, m, H-2)/115.3 (C-2), 2.45 (1H, q, *J* = 8.4 Hz, H-2′)/50.4 (C-2′) and 1.98 (1H, m, H-4′)/36.2 (C-4′)), and four quaternary carbons (δ_C 213.2 (C-1′), 167.1 (C-1), 160.2 (C-3) and 43.4 (C-3′)). Among these signals, the characteristic values at δ_C 213.2 (C-1′), 167.1 (C-1), 160.2 (C-3), 115.3 (C-2) and 50.9 (O<u>C</u>H$_3$) showed the presence of a ketone, a methyl ester, an olefin and a methoxyl group, respectively. The connectivity of the protonated carbons including C-4/C-5, C-9′/C-4′/C-5′/C-6′ and C-2′/C-7′ was determined by the ^{1}H–^{1}H COSY experiment. The HMBC analysis indicated the key correlation peaks from δ_H 2.34 (H-6′) and 0.91 (H-7′) to 213.2 (C-1′), from 0.58 (H-8′) to 36.2 (C-4′), and from 0.88 (H-9′) to 43.4 (C-3′) (Fig. 2). These results suggested that **3** included a 2′,3′,4′-trimethyl-1′-cyclohexanone derivative in its structure. The relative stereochemistry was determined by the cross-linked peaks of H-7′/H-8′ and H-8′/H-9′ in the NOESY spectrum (Fig. 3). This assignment indicated that methyl groups at C-3′ and C-4′ were, respectively, axial and equatorial configurations. The absolute configuration was identified as 2′*R*, 3′*S*, 4′*R* by comparison with the rotation value ($[\alpha]^{20}_D$ +3.86, *c* 0.50, MeOH) in the literature (Zhang et al., 2009). The spectroscopic data for the other parts of **3** were similar to those for **1** and **7**–**9** which all contain a methyl 3-methyl-2*E*-pentenoate moiety. The linkage of the two units was determined by the cross-peak from δ_H 1.44 (H-5) to δ_C 36.2 (C-4′), 43.4 (C-4) and 50.4 (C-2′) in the HMBC spectrum. Consequently, compound **3** was named (2*E*)-3-methyl-5-

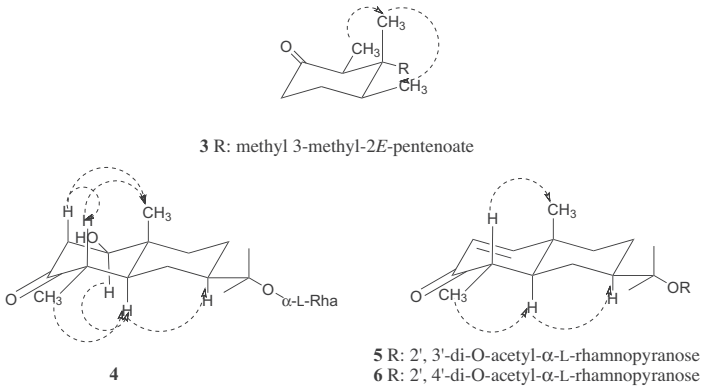

Fig. 3. The NOESY correlations of compounds **3–6**.

[(1′*S*,2′*R*,6′*R*)-1′,2′,6′-trimethyl-3′-oxocyclohexyl]-pent-2-enoic acid methyl ester.

Compound **4** was a brownish syrup with the molecular formula, $C_{21}H_{36}O_7$, as determined by the quasi-molecular ion peak at *m/z* 423.2343 $[M + Na]^+$ (calcd. for 423.2353) obtained by HRESIMS. In the ^1H, ^{13}C NMR and HSQC spectra, **4** has characteristic signals of a ketone group at δ_C 213.2 (C-3), five oxygenated methine groups at δ_H/δ_C 3.41 (1H, dd, J = 5.0, 12.0 Hz, H-1)/79.7 (C-1), 3.65 (2H, m, H-3′ and 5′)/74.5 (C-3′) and 70.9 (C-5′), 3.60 (1H, dd, J = 3.2, 9.4 Hz, H-2′)/73.4 (H-2′) and 3.34 (1H, m, H-4′)/74.8 (C-4′), five methyl groups at δ_H/δ_C 1.28 (3H, s, H-13)/27.1 (C-13), 1.26 (3H, s, H-12)/25.8 (C-12), 1.18 (3H, d, J = 6.3 Hz, H-6′)/18.7 (C-6′), 1.07 (3H, s, H-14)/12.6 (C-14) and 0.98 (3H, d, J = 6.5 Hz, H-15)/12.6 (C-15), and an anomeric carbon at δ_H/δ_C 4.96 (3H, s, H-1′)/97.1 (C-1′) of a sugar residue. The connectivity of H-1/H-2 and H-15/H-4/H-5/H-6/H-7/H-8/H-9 was determined from a ^1H–^1H COSY spectrum. The HMBC spectrum indicated that the structure of **4** consisted of the aglycone of a eudesmane sesquiterpenoid and a sugar residue (Fig. 2). The correlation peaks from H-2 at δ_H 2.59 (1H, t, J = 13.4 Hz) and 2.46 (1H, dd, J = 5.2, 13.4 Hz), and H-14 at δ_H 1.07 (3H, s) to C-1 at δ_C 79.7, and from H-2 and H-15 at δ_H 0.98 (1H, d, J = 6.5 Hz) to C-3 at δ_C 213.2 suggested that a hydroxyl moiety and a ketone group were present at C-1 and C-3, respectively. The anomeric proton at δ_H 4.96 (H-1′) had a cross-peak with the signal of C-11 at δ_C 82.4 and this result indicated the sugar residue was at C-11. The type and configuration of the sugar moiety were α-rhamnose as determined by comparison of the coupling constants and chemical shifts with previously reported data (Agrawal, 1992). After acid hydrolysis of **4**, it was unequivocal, based on UPLC-QTOF/MS analysis that the type of sugar residue was L-rhamnose. The NOESY analysis established the relative stereochemistry of **4** by the presence of the correlation peaks of H-2β/H-4/H-6β/H-14, H-6α/H-11 and H-1/H-5/H-7 (Fig. 3). The splitting pattern (dd) and the coupling constants of H-1 (J = 5.0, 12.0 Hz) indicated that the hydroxyl moiety at C-1 is β-equatorial, not α-axial, based on a comparison with those of stereoisomers that are α- or β-side oriented (Yamakawa and Nishitani, 1976; Zhu et al., 2010). Based on the above spectroscopic data, compound **4** was identified as 1β-hydroxy-3-oxo-4β,5α,7α-H-eudesmane 11-*O*-α-L-rhamnopyranoside.

Compound **5** was obtained as a pale yellow syrup. The molecular formula $C_{25}H_{38}O_8$ was established by the positive HRFABMS (*m/z* 467.2650 $[M+H]^+$, calcd. for 467.2645). The ^1H and ^{13}C NMR, and HSQC spectra suggested the presence of a ketone at δ_C 205.4 (C-3), an olefinic carbon at δ_H/δ_C 6.81 (1H, d, J = 9.9 Hz, H-1)/164.9 (C-1) and 5.72 (1H, d, J = 9.9 Hz, H-2)/127.1 (C-2), two acetoxy carbons at δ_C 172.7 (C-1″) and 172.6 (C-1‴), five tertiary methyl carbons at δ_H/δ_C 1.14 (3H, s, H-14)/18.5 (C-14), 1.13 (3H, d, J = 6.3 Hz, H-15)/12.8 (C-15), 1.32 (3H, s, H-12)/25.9 (C-12), 1.33 (3H, s, H-13)/27.3 (C-13), 1.08 (3H, d, J = 6.3 Hz, H-6′)/18.4 (C-6′) and an anomeric carbon at δ_C 96.8 (C-1′). The long chain of H-4/H-5/H-6/H-7/H-8/H-9 was identified by using the ^1H-^1H COSY experiment. According to the HMBC analysis, the correlation peaks from δ_H 1.14 (H-14) to δ_C 164.9 (C-1), 37.8 (C-10) and 36.6 (C-9), and from δ_H 1.13 (H-15) to δ_C 205.4 (C-3), 45.5 (C-5) and 44.8 (C-4) indicated the two methyl carbons attached to C-10 and C-4, respectively. The presence of an α, β-unsaturated ketone was deduced from the characteristic chemical shifts of δ_C 205.4 (C-3), 164.9 (C-1) and 127.1 (C-2), and their cross-peaks from H-1 to C-3. In addition, the HMBC data showed that two methyl carbons at C-12 and C-13 attached to an oxygenated quaternary carbon at δ_C 83.4 (C-11), were correlated with a methine carbon at δ_C 44.2(C-7) (Fig. 2). These results indicated that an isopropyl alcohol moiety was located at C-7. In the NOESY spectrum, the correlations of H-4/H-14 and H-5/H-7/H-15 indicated the relative configuration of **5** (Fig. 3). The absolute configuration of the aglycone of **5** was assumed to have the structure of 4-*epi*-aubergenone (Kelly et al., 1978; Murai et al., 1982; Uegaki et al., 1988). Location of the sugar residue was deduced to be at C-11, on the basis of the long range correlation from δ_H 5.00 (H-1′) to δ_C 83.4 (C-11). The coupling constant of an anomeric proton at δ_H 5.00 (1H, s, H-1′) indicated the sugar had an α-orientation (Barrero, 1997). By the responses at δ_H/δ_C 1.08 (1H, d, J = 1.1 Hz, H-6′)/18.4 (C-6′) which was connected to C-5′, the type of sugar residue was determined to be an L-rhamnose. Additionally, the characteristic signals at δ_H/δ_C 2.00 (3H, s, H-2″)/21.6 (C-2″) and 1.99 (3H, s, H-2‴)/21.5 (C-2‴), and at δ_C 172.7 (C-1″) and 172.6 (C-1‴) indicated the presence of two acetoxy moieties. It was confirmed that they were unequivocally located at C-2′ and C-3′ in a sugar residue, based on the cross-peaks from δ_H 3.82 (H-2′) to δ_C 172.7 (C-1″), and from δ_H 4.95 (H-3′) to δ_C 172.6 (C-1‴), respectively. These results confirmed that the sugar residue was 2,3-di-*O*-acetyl-α-L-rhamnopyranose (Pozsgay and Neszmélyi, 1980). Consequently, the structure of **5** was determined as 4-*epi*-aubergenone 11-*O*-2′, 3′-di-*O*-acetyl-α-L-rhamnopyranoside.

Compound **6** was obtained as a pale yellow syrup. Its molecular formula of $C_{25}H_{38}O_8$ was established on the basis of the positive HRFABMS (*m/z* 467.2644 $[M+H]^+$, calcd. for 467.2645). In a comparison of the ^1H, ^{13}C NMR, and HSQC spectra of **6** with those of **5**, the formed appeared to have an identical structure apart from the sugar residue. Structure **6** differed from that of **5** in the signals of a sugar residue attached to two acetoxy groups. According to the HMBC data, the sugar residue was identified as 2, 4-di-*O*-acetyl-α-L-rhamnopyranose by the correlation peaks from δ_H 4.78 (H-2′) to 172.7 (C-1″) and from δ_H 4.85 (H-4′) to δ_C 172.6 (C-1‴) (Fig. 2). Compound **6** was named 4-*epi*-aubergenone 11-*O*-2′,4′-di-*O*-acetyl-α-L-rhamnopyranoside.

UPLC-QTOF/MS analysis was performed to determine the identity of the components in the CH_2Cl_2 and *n*-BuOH fractions (see Supplementary data). The retention times and MS spectra of the peaks detected in the chromatograms were compared to those of the isolated compounds in order to identify the components present in the extract of *C. latifolia*. Interestingly, UV and MS analyses showed that JH III (**7**), (2*E*,6*E*,10*R*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoaic acid methyl ester (**8**) and (2*E*,6*E*,10*R*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoaic acid methyl ester (**9**) were present in relatively large amounts in the extract of *C. latifolia* stem bark.

3. Concluding remarks

There are many examples of the presence of insect hormone analogues, such as phytoecdysteroids, in plants. However, JH III (**7**), which is secreted by the corpora allata behind the brain in insects, has only been reported to be present in *Cyperus* species. In the present study, we succeeded in isolating it from the stem bark of *C. latifolia*. In addition, six new compounds including two linear sesquiterpenes (**1**–**2**) and four cyclic sesquiterpenes (**3**–**6**) were isolated, it was also ascertained, by using HPLC-UVD-ESI/MS analysis, that it analogues were abundant in an 80% MeOH extract of the stem bark of *C. latifolia*. Since JH III analogues are relatively harmless to non-arthropods but are selectively effective on insects, they have been used in the development of natural insecticides, such as Gentrol® and Precor®. Our results strongly suggest the potential of using extracts of *C. latifolia*, or its isolated active compounds, in the development of natural insecticides.

4. Experimental

4.1. General experimental procedure

Column chromatography (CC) was accomplished by Kiesgel 60 silica gel (40–60 μm, 230–400 mesh, Merck, USA), YMC-GEL ODS-A (5–150 μm, YMC) and Sephadex LH-20 (25-100 μM, Pharmacia, NJ, USA), and TLC was carried out on Kiesslgel 60 F_{254} coated normal silica gel and RP-18 F_{254} coated reverse-phase silica gel. The HPLC system consisted of a G-321 pump (Gilson Co. Ltd., USA), a G-151 UV detector (Gilson Co. Ltd., USA) and a Kromacil C_{18} column (250 mm × 21.2 mm i.d.; 5 μm), and all chromatograms were monitored at 210 nm. HPLC grade solvents (Fisher Scientific, USA) were used in the CH_3CN–H_2O system. 1D and 2D NMR spectra were recorded on Bruker AMX 400 or 500 spectrometers. FT-IR spectra were measured with a JASCO FT/IR-300 spectrophotometer. High-resolution and low-resolution FABMS were obtained on a JEOL JMS-AX505WA. HPLC/ESI-MS mass spectrometry analyses were performed using a Finnigan Surveyor MS Pump Plus and Finnigan LCQ Advantage Max connected to an Ascenti C_{18} column (150 mm × 4.6 mm i.d.; 3 μm).

4.2. Plant material

C. latifolia identity was confirmed by Prof. Thoa Sokunthea, Head, Department of Biology, Faculty of Science, Royal University of Phnom Penh, Cambodia. It was first purchased in January 2011, and then was collected in September 2010 in Kompong Speu province, Cambodia. Voucher specimen (SNUPH-0426) is deposited at the Herbarium in the Medicinal Plant Garden, Seoul National University.

4.3. Extraction and isolation procedure

A dried sample of *C. latifolia* (960 g) was extracted with MeOH–H_2O (4 L × 3, 4:1, v/v) using an ultrasonic apparatus (3 h × 3). The total extract (125 g) which was evaporated *in vacuo* was fractionated with CH_2Cl_2 (15 g) and *n*-BuOH (20 g), sequentially. The CH_2Cl_2 fraction was subjected to reversed phase silica CC eluting with a gradient of MeOH:H_2O (1:9, 5:5, 7:3, 9:1, 10:0, each 1 L) to give five sub-fractions (C1–C5). Of these, C2 was further separated into nine fractions (C2-1–9, each 500 mL) on MPLC using mixtures of $CHCl_3$–MeOH of increasing polarity ($CHCl_3$–MeOH = 1:9, 5:5, 5 L). Compounds **13** (26.1 mg) and **14** (56.3 mg) were isolated from C2-5 fraction using a MPLC system eluting with mixtures of H_2O–MeOH (5:5, 2:8, each 100 mL). Compound **4** (13.4 mg) in C2-8 fraction was crystallized from MeOH in the dark. C3 was subjected to HPLC (H_2O–CH_3CN = 5:5, 1:9, 10 mL/min, 40 min) and yielded compound **11** (5.7 mg, t_R 19.1 min). By MPLC (H_2O–CH_3CN = 5:5, 3:7, 6 L), fraction C4 was further separated into 13 sub-fractions (C4-1–13). From these sub-fractions, an HPLC system eluted with the mixtures of H_2O–CH_3CN yielded compounds **7** (20.1 mg, 25:75, 10 mL/min, t_R 10.9 min from C4-1), **1** (3.7 mg, 3:7, 10 mL/min, t_R 21.3 min from C4-2), **3** (3.9 mg, 2:8, 10 mL/min, t_R 17.2 min from C4-3), **9** (13.9 mg, 3:7, 10 mL/min, t_R 8.2 min from C4-4), **2** (2.0 mg, 3:7, 10 mL/min, t_R 18.4 min from C4-7), **5** (9.9 mg, 1.5:8.5, 10 mL/min, t_R 12.5 min from C4-8), and **6** (20.9 mg, 1.5:8.5, 10 mL/min, t_R 14.1 min from C4-8), respectively. Compound **15** was recrystallized with MeOH from the C4-13 fraction. The *n*-BuOH fraction was divided into nine sub-fractions (Bu1-9) by normal silica CC using mixtures of $CHCl_3$–MeOH of increasing polarity (100:1, 50:1, 10:1, 5:1, 0:1, each 1 L). Bu2 fraction was subjected to MPLC equipped with a reversed phase silica CC (H_2O–MeOH = 8:2, 1:9, each 400 mL) and yielded compound **8** (482.0 mg). Compounds **10** (6.1 mg, H_2O–MeOH = 8:2, 1:9, each 200 mL) and **12** (8.8 mg, H_2O–MeOH = 9:1, 0:10, each 200 mL) were obtained from Bu3 and Bu9 fractions using an MPLC system, respectively.

4.4. (2E,6E,10R)-11-butoxy-10-hydroxy-3, 7,11-trimethyldodeca-2,6-dienoic acid methyl ester (*1*)

Colorless syrup; $[\alpha]^{20}_D$ +16.7 (*c* 0.2, MeOH); IR (KBr) ν_{max}: 3450, 2948, 2931, 2857, 1719, 1648, 1436, 1383 1358 cm^{-1}; for ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1; FABMS (positive ion mode) *m*/*z* 341 $[M+H]^+$; HRFABMS (positive ion mode) *m*/*z* 341.2690 $[M+H]^+$ (calcd. for $C_{20}H_{37}O_4$, 341.2692).

4.5. (2E,6E)-3,7,11-trimethyl-10-oxododeca-2,6-dienoic acid methyl ester (*2*)

Pale yellow syrup; $[\alpha]^{20}_D$ +1.2 (*c* = 0.5, MeOH); IR (KBr) ν_{max}: 2970, 2935, 1710, 1693, 1438, 1383 cm^{-1}; for ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data, see Table 1; FABMS (positive ion mode) *m*/*z* 253 $[M+H]^+$; HRESIMS (positive ion mode) *m*/*z* 253.1791 $[M+H]^+$ (calcd. for $C_{15}H_{26}O_3$, 253.1798).

4.6. (2E)-3-methyl-5-[(1S,2R,6R)-1,2,6-trimethyl-3-oxocyclohexyl]-pent-2-enoic acid methyl ester (*3*)

Pale yellow syrup; $[\alpha]^{20}_D$ +16.2 (*c* 0.7, MeOH); IR (KBr) ν_{max}: 2967, 2950, 1714, 1648 1435, 1379, 1357 cm^{-1}; for ^1H NMR ($CDCl_3$, 400 MHz): δ 5.67 (1H, m, H-2), 3.67 (3H, s, OCH_3), 2.45 (1H, q, *J* = 3.9 Hz, H-2′), 2.34 (2H, m, H-6′), 2.17 (3H, s, H-6), 2.14 (1H, m, H-4a), 1.99 (1H, m, H-4b), 1.98 (1H, m, H-4′), 1.83 (1H, m, H-5′a), 1.62 (1H, m, H-5′b), 1.44 (2H, m, H-5), 0.91 (3H, d,

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1**–**2**.

Position	**1**		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		167.3		171.5
2	5.64 s	115.2	5.66 s	115.1
3		160.1		162.7
4	2.14 m	40.9	2.15 m	25.8
5	2.14 m	25.9	2.14 m	41.0
6	5.12 m	123.1	5.06 s	123.2
7		136.2		135.3
8	2.27 m, 2.00 m	36.8	2.21 t (7.4)	33.4
9	1.45 m	29.6	2.51 t (7.4)	39.0
10	3.36 dd (1.8, 10.2)	76.5		214.5
11		77.0	2.58 septet (7.0)	40.9
12	1.06 s	19.2	1.06 d (7.0)	18.2
13	1.09 s	21.4	1.06 d (7.0)	18.2
14	1.59 s	16.1	1.58 s	16.1
15	2.14 m	18.8	2.14 s	19.1
OCH_3	3.66 s	50.8		
1′	3.33 td (2.2, 6.5)	60.7		
2′	1.48 m	32.6		
3′	1.33 m	19.4		
4′	0.89 t (7.4)	13.9		

^{1}H and ^{13}C NMR data were measured at 500 and 125 MHz in CD_3OD, respectively.

Table 2
^{1}H and ^{13}C NMR spectroscopic data for compounds **4**–**6**.

Position	**4**		**5**		**6**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	3.41 dd (5.0, 12.0)		6.81 d (9.9)	164.9	6.81 d (9.9)	164.3
2	2.59 t (13.4), 2.46 dd (5.0, 13.4)	48.7	5.72 d (9.9)	127.1	5.80 d (9.9)	127.3
3		213.2		205.4		205.2
4	2.15 m	47.5	2.26 m	44.8	2.25 m	44.9
5	1.44 m	44.1	2.31 m	45.5	2.25 m	45.4
6	1.99 m, 1.43 m	27.3	2.21 m, 1.50 m	26.8	2.05 m, 1.50 m	26.8
7	1.73 m	44.3	1.67 m	44.2	1.70 m	43.8
8	1.80–1.61 m	22.4	1.92 m, 1.78 m	22.7	1.90 m, 1.80 m	22.7
9	1.63 m	36.5	1.97 m, 1.41 m	36.6	1.80 m, 1.50 m	36.9
10		40.1		37.8		37.7
11		82.4		83.4		83.7
12	1.26 s	25.8	1.32 s	25.9	1.29 s	26
13	1.28 s	27.1	1.33 s	27.3	1.29 s	27
14	1.07 s	12.6	1.14 s	18.5	1.15 s	18.8
15	0.98 d (6.5)	12.6	1.13 d (6.3)	12.8	1.11 d (6.2)	12.8
1′	4.96 s	97.1	5.00 s	96.8	5.10 s	94.1
2′	3.60 dd (3.2, 9.4)	73.4	3.82 m	71.6	4.78 m	76.1
3′	3.65 m	74.5	4.95 dd (3.0, 10.0)	74.4	3.90 dd (4.4, 12.1)	69.2
4′	3.34 m	74.8	5.03 t (9.9)	73.3	4.85 m	76.4
5′	3.65 m	70.9	3.90 m	68.8	3.80 m	68.7
6′	1.18 d (6.3)	18.7	1.08 d (6.3)	18.4	1.00 d (6.3)	18.5
1″				172.7		172.7
1‴				172.6		172.6
2″			2.00 s	21.6	2.15 s[a]	21.6
2‴			1.99 s	21.5	2.05 s[a]	21.5

^{1}H and ^{13}C NMR data were measured at 400 and 100 MHz in CD_3OD, respectively.
[a] Assignment may be interchanged, though it given here is preferred.

J = 6.7 Hz, H-7′), 0.88 (3H, d, J = 6.8 Hz, H-9′), 0.58 (3H, s, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 213.2 (C-1′), 167.1 (C-1), 160.2 (C-3), 115.3 (C-2), 50.9 (OCH_3), 50.4 (C-2′), 43.4 (C-3′), 41.5 (C-6′), 36.2 (C-4′), 35.2 (C-5), 34.1 (C-4), 30.8 (C-5′), 19.1 (C-6), 15.3 (C-8′), 15.0 (C-9′), 7.6 (C-7′); ESIMS (positive ion mode) m/z 267 $[M+H]^+$; HRESIMS (positive ion mode) m/z 267.1948 $[M+H]^+$ (calcd. for 267.1955).

4.7. 1β-Hydroxy-3-oxo-4β, 5α,7α-H-eudesmane 11-O-α-L-rhamnopyranoside (**4**)

Brownish syrup; $[\alpha]^{20}_D$ +4.3 (c 1.0, MeOH); IR (KBr) ν_{max}: 3404, 2972, 2934, 1702, 1601, 1514, 1454, 1386 cm^{-1}; for ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 2; ESIMS (positive ion mode) m/z 423 $[M+Na]^+$; HRESIMS (positive ion mode) m/z 423.2343 $[M+Na]^+$ (calcd. for 423.2353).

4.8. 4-Epi-aubergenone 11-O-2′,3′-di-O-acetyl-α-L-rhamnopyranoside (**5**)

Pale yellow syrup; $[\alpha]^{20}_D$ +4.8 (c 1.0, MeOH); IR (KBr) ν_{max}: 3471, 2978, 2938, 1744, 1675, 1455, 1372 cm^{-1}; For ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 2; FABMS (positive ion mode) m/z 467 $[M+H]^+$; HRFABMS (positive ion mode) m/z 467.2650 $[M+H]^+$ (calcd. for 467.2645).

4.9. 4-Epi-aubergenone 11-O-2′,4′-di-O-acetyl-α-L-rhamnopyranoside (**6**)

Pale yellow syrup; $[\alpha]^{20}_D$ +2.7 (c 1.0, MeOH); IR (KBr) ν_{max}: 3471, 2978, 2938, 1744, 1675, 1455, 1373 cm^{-1}; for ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see Table 2; FABMS (positive ion mode) m/z 467 $[M+H]^+$; HRFABMS (positive ion mode) m/z 467.2644 $[M+H]^+$ (calcd. for 467.2645).

4.10. General acid hydrolysis

Compound **4** (2 mg) was reacted with 1 N HCl (dioxane-H_2O, 1:1, 1 mL) and deposited in a dry oven (60 °C). After 2 h, the solution was evaporated to dryness under N_2 and fractionated with $CHCl_3$ and H_2O. The aqueous layer was concentrated to dryness. The type of sugar in **4** was compared with a L-rhamnose standard (Sigma Aldrich, St. Louis, USA) by UPLC-QTOF/MS analysis (Xevo-G2 + QTOF system; Waters Acquity BEH Amide, 100 × 2.1 mm 1.7 μm; Waters Corp., Milford, MA, USA). The sample and standard were separated using a by the gradient solvent condition consisting of 30% CH_3N (A) and 80% CH_3N (B) (0% (A, 0 min), 100% (A, 8 min)). The retention time of L-rhamnose standard was 3.84 min.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.06.010.

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