CHAPTER IV

RESULTS AND DISSCUSION

The pulverized leaves and stems of *Ellipeiopsis cherrevensis* (Pierre ex Finet & Gagnep.) R. E. Fr. were separately extracted with organic solvents to give hexane, $CHCl_3$ and MeOH extracts of the leaves and stems. Separation of the extracts from the leaves by several chromatographic techniques afforded seven compounds (EC-1, EC-2, EC-3, EC-4, EC-5, EC-6 and EC-7), whereas the extracts of the stems yielded two compounds (EC-5 and EC-7).

The dried, ground leaves and stems of *Stelechocarpus cauliflorus* R. E. Fr. were separately macerated with hexane, EtOAc and MeOH, to give hexane, EtOAc and MeOH extracts of the leaves and stems, respectively. Six compounds (SC-1, SC-2, SC-3, SC-4, SC-5 and SC-6) were isolated from the leaf extracts. The extracts from the stems were extensively chromatographed to yield four compounds (SC-8, SC-9, SC-10, SC-11) and a mixture of two stereoisomers (SC-7).

The structures of all isolated compounds were identified and elucidated by interpretation of their UV, IR, MS and NMR spectral data, and confirmed by comparison with the literatures.

1. Structure Determination of Compounds Isolated from *Ellipeiopsis cherrevensis*

1.1 Identification of Compound EC-1

Compound EC-1 was obtained as white needle crystals, soluble in $CHCl_3$. Its UV spectrum (**Figure 10**) exhibited absorption maxima at 243 and 276 nm, indicating the presence of benzoyl group(s). The IR spectrum (**Figure 11**) showed absorption bands of hydroxy group at 3449 cm^{-1}, ester carbonyl at 1716 cm^{-1}, and substituted phenyl rings at 1272, 1112 and 712 cm^{-1}. The CI mass spectrum (**Figure 12**) displayed $[M+H]^+$ ion peak at *m/z* 489, corresponding to a molecular formula of $C_{28}H_{24}O_8$. The CI-MS also gave mass fragment peak at *m/z* 471 ($[M+H-H_2O]^+$), confirming the presence of at least one hydroxy group.

The ^{13}C NMR spectrum of compound EC-1 (**Figure 14**) displayed four signals of methine carbons bearing oxygen in the aliphatic region at δ 72.9 (C-6), 75.5 (C-2), 76.1 (C-1) and 76.7 (C-3), one methylene carbon bearing oxygen (δ 62.7, C-7), two

olefinic carbons at δ 127.9 (C-4) and 128.3 (C-5), three ester carbonyl carbons at δ 166.5 (C-7″), 166.6 (C-7′) and 167.2 (C-7‴), while the rest were those of three aromatic rings.

The proton NMR spectrum (**Figure 13**) showed two olefinic protons of a cyclohexene ring at δ 5.98 (H-4) and 5.86 (H-5), with typical coupling constant of 10.3 Hz, indicating *cis* conformation. The carbinyl methylene protons of position 7 appeared separately as doublets (J = 12.0 Hz) at δ 4.80 and 4.74. In addition, a doublet at δ 4.29 (1H, *d*, J = 8.5 Hz) was assigned as H-2 by analysis of the ^{1}H-^{1}H COSY spectrum (**Figure 16**). The large magnitude of coupling constant between H-2 and H-3 (J = 8.5 Hz), indicated a *trans* pseudo-diaxial arrangement of H-2/H-3.

The ^{1}H NMR spectrum also showed the resonances of three benzoate groups at δ7.32 – 8.07, confirmed by mass fragments at *m/z* 367 ($[M+H-PhCOOH]^{+}$), 245 ($[M+H-2PhCOOH]^{+}$), 123 ($[M+H-3PhCOOH]^{+}$) and 105 ($[PhCO]^{+}$), due to the loss of these benzoyloxy groups. The three benzoyloxy groups were clearly located at positions 3, 6 and 7 of the cyclohexene ring, judging from the downfield shifts of the proton signals of these positions. Long-range HMBC correlations (**Figure 18**) observed between the signals of H-7 and C-7′ (δ 166.6), H-6 and C-7‴ (δ 167.2) and H-3 and C-7″ (δ 166.5) further confirmed these assignments.

The NOESY correlations (**Figure 19**) between H-7 and H-3, as well as between H-2 and H-6, suggested the relative conformation at positions 2, 3 and 6. Therefore compound EC-1 was identified as ferrudiol, a cyclohexene derivative which was isolated from two plants of the family Annonaceae: *Uvaria ferruginea* (Schulte *et al.*, 1982) and *Ellipeiopsis cherrevensis* (Kijjoa *et al.*, 2002). However, no bioactivity of this compound has been reported.

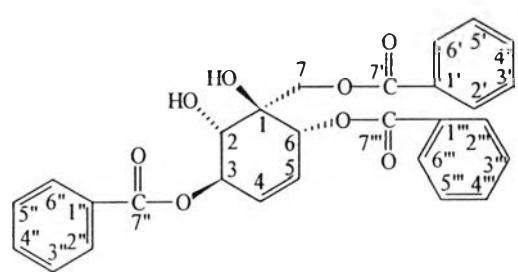

Table 17. Comparison of the ^{1}H and ^{13}C NMR spectral data of ferrudiol and compound EC-1 (in $CDCl_3$, 500 MHz)

Position	Ferrudiol†		Compound EC-1	
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	76.0	-	76.1
2	4.31 (1H, *d*, *J* = 8.5 Hz)	75.4	4.29 (1H, *d*, *J* = 8.5 Hz)	75.5
3	5.82 – 5.85 (1H, *m*)	76.8	5.80 – 5.84 (1H, *m*)	76.7
4	6.01 (1H, *dt*, *J* = 10.2, 1.6 Hz)	127.9	5.98 (1H, *dt*, *J* = 10.3, 2.0 Hz)	127.9
5	5.88 (1H, *dt*, *J* = 10.2, 1.6 Hz)	128.3	5.86 (1H, *dt*, *J* = 10.3, 2.0 Hz)	128.3
6	5.82 – 5.85 (1H, *m*)	72.8	5.80 – 5.84 (1H, *m*)	72.9
7	4.83 (1H, *d*, *J* = 12.0 hz)	62.9	4.80 (1H, *d*, *J* = 12.0 Hz)	62.7
	4.75 (1H, *d*, *J* = 12.0 hz)		4.74 (1H, *d*, *J* = 12.0 Hz)	
1′	-	129.5	-	129.6*
2′, 6′	7.99 (2H, *dd*, *J* = 8.5, 1.5 Hz)	129.9	8.07 (1H, *dd*, *J* = 8.0, 1.5 Hz)	129.9
3′, 5′	7.47 (2H, *tt*, *J* = 8.0, 1.5 Hz)	128.5	7.44 (2H, *tt*, *J* = 7.8, 1.2 Hz)	128.4
4′	7.60 (1H, *tt*, *J* = 7.5, 1.5 Hz)	133.4	7.57 (1H, *tt*, *J* = 7.8, 1.2 Hz)	133.4
7′	-	166.6	-	167.2
1″	-	129.3	-	129.4*
2″, 6″	8.10 (2H, *dd*, *J* = 8.5, 1.5 Hz)	130.0	7.97 (2H, *dd*, *J* = 7.8, 1.2 Hz)	130.0
3″, 5″	7.34 (2H, *tt*, *J* = 7.5, 1.5 Hz)	128.4	7.32 (2H, *tt*, *J* = 8.0, 1.5 Hz)	128.5
4″	7.52 (1H, *tt*, *J* = 7.5, 1.5 Hz)	133.1	7.49 (1H, *tt*, *J* = 8.0, 1.5 Hz)	133.6
7″	-	166.5	-	166.6
1‴	-	128.9	-	129.0*
2‴, 6‴	7.91 (2H, *dd*, *J* = 8.5, 1.5 Hz)	129.6	7.90 (1H, *dd*, *J* = 8.0, 1.0 Hz)	129.7
3‴, 5‴	7.32 (2H, *tt*, *J* = 7.5, 1.5 Hz)	128.3	7.30 (2H, *tt*, *J* = 8.0, 1.0 Hz)	128.3
4‴	7.47 (1H, *tt*, *J* = 8.0, 1.5 Hz)	133.1	7.47 (1H, *tt*, *J* = 8.0, 1.0 Hz)	133.1
7‴	-	167.2	-	166.5

† Kijjoa *et al.*, 2002 (in $CDCl_3$, 500 MHz)

1.2 Structure Elucidation of Compound EC-2

Compound EC-2 was obtained as orange needle crystals. Its molecular formula was determined by HREIMS (**Figure 22**) as $C_{23}H_{20}O_5$, from its $[M]^+$ ion peak at *m/z* 376.1311. The UV absorption maxima (**Figure 21**) at 243 and 347 nm, and the IR absorption band (**Figure 21**) at around 3272 cm^{-1} (OH group), together with the band at 1627 cm^{-1} (conjugated carbonyl) were indicative of compound EC-2 as a chalcone (Markham, 1982).

The ^{13}C NMR spectrum of compound EC-2 (**Figure 24**) displayed twenty-three carbon signals, corresponding to one methoxy, one carbonyl, one methylene, twelve methine and eight quaternary carbons.

The ^1H NMR spectrum (**Figure 23a** and **23b**) showed the characteristic *trans* double bond signals at δ 8.04 and 7.77 (both 1H, d, J = 15.6 Hz, H-α and H-β, respectively), and also exhibited a methoxy singlet at δ 3.95. A chelated hydroxy proton resonance at δ 15.04 and a carbon signal at δ 193.2 were consistent with a 2′-hydroxychalcone skeleton. The C-benzylated nature of this chalcone could be determined from the number of aromatic proton and carbon signals and a singlet resonance of the benzylic methylene protons appearing at δ 3.90 (H-7″). The proton spectrum exhibited eight aromatic proton resonances integrated for 10 protons. The ^1H-^1H COSY experiment (**Figure 26**) was used to classified each groups of these aromatic protonsinto those of the monosubstituted ring B (δ 7.72, 7.45 and 7.45), the pentasubstituted ring A (δ 6.19) and a 1,2-disubstituted aromatic ring (δ 6.82, 6.99, 6.73, and 7.25).

Comparison of the NMR spectral data of compound EC-2 with those previously reported for other C-benzylated chalcones revealed its ring substitution patterns to closely resemble those of 2′,4′-dihydroxy-3′-(2,6-dihydroxybenzyl)-6′-methoxychalcone (Rahman *et al.*, 2003) isolated from *Desmos chinensis*. Detailed analysis of the data indicated that the difference in compound EC-2 was its benzyl moiety, which was substituted by one hydroxy group at position 2″ only. Therefore, compound EC-2 was identified as 2′,4′-dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone, previously reported as an intermediate product in the chemical synthesis of uvaretin, an antitumour and antimicrobial dihydrochalcone found in several *Uvaria* species (Malterud *et al.*, 1985). However, this is the first report of its occurrence in nature.

C-Benzylated dihydrochalcones can be found in several *Uvaria* species of the Annonaceae. However, C-benzylated chalcones appear to be rare in nature and only one compound of this type has previously been reported from *Desmos chinensis,* which is another annonaceous plant.

These C-benzylated chalconoids were shown to exhibit cytotoxic activity e.g. uvaretin and isouvaretin were cytotoxic against P-388 leukemia cells in the mouse and *in vitro* against human carcinoma of the nasopharynx (KB) cell line (Cole, *et al.*, 1976; Hufford and Lasswell, Jr., 1976). Two other C-benzylated chalconoids, anguvetin and angoluvarin, exhibited antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Mycobacterium smegmatis* (Hufford and Oguntimein, 1982; 1987), whereas uvaretin and diuvaretin exhibited antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 3.49 and 4.20 μg/ml, respectively (Khunya *et al.*, 1991).

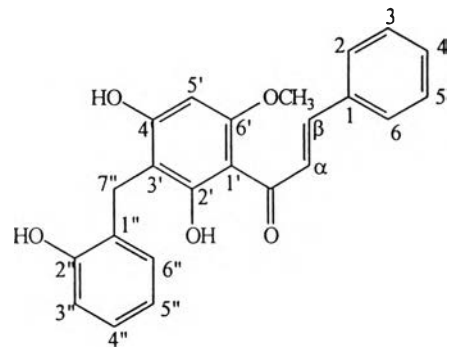

Table 18. The ^{1}H and ^{13}C NMR assignments of 2′,4′-dihydroxy-3′-(2-hydroxybenzyl)- 6′-methoxychalcone (compound EC-2) (in acetone-d_6)

Position	Compound EC-2		
	^{1}H	^{13}C	HMBC
1	-	136.5	
2,6	7.72 (2H, *dd*, *J* = 8.2, 1.8 Hz)	129.8	C-4
3,5	7.45 (2H, *m*)	129.2	C-3, C-5
4	7.45 (1H, *m*)	130.9	C-2, C-6
α	8.04 (1H, *d*, *J* = 15.6 Hz)	128.5	C-1
β	7.77 (1H, *d*, *J* = 15.6 Hz)	142.7	C-2, C-6
C=O	-	193.2	
1′	-	108.1	
2′	-	166.4	
3′	-	106.1	
4′	-	164.2	
5′	6.19 (1H, *s*)	92.4	C-1′, C-3′
6′	-	162.6	
1″	-	127.9	
2″	-	155.2	
3″	6.82 (1H, *dd*, *J* = 7.6, 1.2 Hz)	116.0	C-1″, C-5″
4″	6.99 (1H, *dt*, *J* = 7.6, 1.2 Hz)	127.8	C-3″, C-6″
5″	6.73 (1H, *dt*, *J* = 7.6, 1.2 Hz)	120.6	C-1″, C-3″
6″	7.25 (1H, *dd*, *J* = 7.6, 1.2 Hz)	131.2	C-4″
7″	3.90 (2H, *s*)	22.9	C-1″, C-6″ C-2′, C-4′
6′-OCH_3	3.95 (3H, *s*)	56.3	C-6′
OH	15.04 (1H, *s*)	-	

1.3 Structure Elucidation of Compound EC-3

Compound EC-3 was obtained as white amorphous powder, soluble in $CHCl_3$. Its UV absorption maxima at 243 and 275 nm (**Figure 30**) were indicative of the presence of benzoyl group. Its IR spectrum (**Figure 31**) suggested the presence of hydroxy group (3464 cm^{-1}), ester carbonyl (1722 cm^{-1}) and monosubstituted phenyl ring (1602, 1453 and 712 cm^{-1}). The molecular formula of compound EC-3, $C_{18}H_{20}O_8$, was determined by high resolution EI mass spectrometry (**Figure 32**).

The ^{1}H-NMR spectrum (**Figure 33**) of compound EC-3 displayed signals of the five protons of a monosubstituted phenyl ring at δ 7.42 (2H, *dd*, J = 7.5, 1.0 Hz, H-3′ and H-5′), 7.55 (1H, *m*, H-4′) and 7.98 (2H, *dd*, J = 8.0, 1.5 Hz, H-2′ and H-6′). Two sharp singlets at δ 2.03 and 2.05, each integrating for three protons, were assignable to two acetoxy methyl groups. Two olefinic proton signals at δ 5.84 (1H, *dd*, J = 10.0, 2.0 Hz, H-4) and 5.86 (1H, *ddd*, J = 10.0, 4.5, 2.0 Hz, H-5) with HMQC correlations (**Figure 37**) to ^{13}C-NMR signals (**Figure 34**) at δ 128.8 (C-4) and 129.5 (C-5), respectively, represent the double bond between positions 4 and 5 within the cyclohexene ring.

A methine proton bearing hydroxy group appeared as a doublet at δ 4.04 (J = 7.0 Hz, H-2), whereas the two acetoxyl groups could be established at C-3 and C-6 based on the downfield shifts of the signals of H-3 (δ 5.49) and H-6 (δ 5.44). Long-range HMBC correlations (**Figure 38**) between both H-7 methylene protons (at δ 4.46 and 4.76) to carbonyl carbon signal of the benzoate (at δ 167.0), and comparison with previously reported data, confirmed that the benzoate was at the usual C-7 position.

The relative stereochemistry of compound EC-3 was established from its large coupling value between H-2 and H-3 (J = 7.0 Hz), indicating *trans* pseudo-diaxial arrangement of these two protons, while the large $J_{5,6}$ value (4.3 Hz) indicated H-6 as pseudoequatorial and the substituents at C-3 and C-6 were *cis* (Jolad *et al.*, 1981). Therefore, compound EC-3 was elucidated as the new 6-acetate analog of ellipeiopsol A (Kijjoa *et al.*, 2002) and was named ellipeiopsol D.

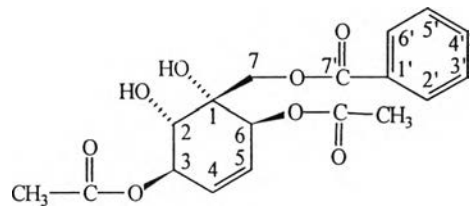

Table 19. The ^{1}H and ^{13}C NMR assignments of ellipeiopsol D (compound EC-3) (in $CDCl_3$, 500 MHz)

Position	^{1}H	^{13}C	HMBC
1	-	74.7	
2	4.04 (1H, *d*, *J* = 7.0 Hz)	71.3	C-3
3	5.49 (1H, *m*)	73.1	C-2, C-4, C-5, 3-$CH_3\underline{CO}$
4	5.79 (1H, *dd*, *J* = 10.0, 2.0 Hz)	128.8	C-6
5	5.86 (1H, *ddd*, *J* = 10.0, 4.3, 2.0 Hz)	125.9	C-1, C-3
6	5.44 (1H, *d*, *J* = 4.3 Hz)	70.2	C-1, C-2, C-4, C-5, 6-$CH_3\underline{CO}$
7	4.76 (1H, *d*, *J* = 12.0 Hz)	66.5	C-1, C-2, C-7′
	4.46 (1H, *d*, *J* = 12.0 Hz)		C-1, C-6, C-7′
1′	-	129.8	
2′, 6′	7.98 (2H, *dd*, *J* = 7.8, 1.3 Hz)	129.7	C-2′, C-4′, C-6′, C-7′
3′, 5′	7.42 (2H, *dd*, *J* = 7.8, 1.3 Hz)	128.5	C-1′, C-3′, C-4′, C-5′
4′	7.55 (1H, *m*)	133.4	C-2′, C-6′
7′	-	167.0	
3-$\underline{CH_3}CO$	2.05 (3H, *s*)	21.1	3-$CH_3\underline{CO}$
3-$CH_3\underline{CO}$	-	171.7	
6-$\underline{CH_3}CO$	2.03 (3H, *s*)	21.1	6-$CH_3\underline{CO}$
6-$CH_3\underline{CO}$	-	179.0	

1.4 Identification of Compound EC-4

Compound EC-4 was obtained as white amorphous powder, soluble in $CHCl_3$. The UV spectrum (**Figure 40**) exhibited absorption maxima at 243 and 276 nm. The IR spectrum (**Figure 41**) displayed absorption bands for hydroxyl (3462 cm^{-1}), carbonyl (1679 cm^{-1}), and aromatic moieties (1281, 1119, 712 cm^{-1}). Its molecular formula was determined to be $C_{21}H_{20}O_7$ from the $[M+H]^+$ peak at *m/z* 385 in the CI mass spectrum (**Figure 42**). In addition, other important fragment ions were observed at *m/z* 105 ($[PhCO]^+$) and *m/z* 367 (loss of a hydroxy group).

Preliminary examination of the spectral data suggested that compound EC-4 possess similar polyoxygenated cyclohexene structure to compounds EC-1 and EC-2, except for the difference in the number of benzoyloxy substitutions. In this case, the major fragments in the CI mass spectrum at *m/z* 263 and 141 due to the loss of one and two benzoyloxy groups, respectively, suggested the presence of two benzoate esters as parts of the molecule.

The ^1H NMR spectrum of compound EC-4 (**Figure 43**) exhibited the resonances of three oxygenated methine protons at δ 4.22 (*d*, J = 6.0 Hz, H-2), 4.32 (*d*, J = 4.0 Hz, H-6) and 5.69 (*m*, H-3), a pair of geminal oxygenated methylene protons at δ 4.72 (1H, *d*, J = 12.0 Hz, H-7a) and 4.86 (1H, *d*, J = 12.0 Hz, H-7b), together with two *cis*-olefinic protons at δ 5.84 (*ddd*, J = 10.3, 2.5, 0.5 Hz, H-4) and 5.98 (*ddd*, J = 10.3, 4.0, 2.0 Hz, H-5). The ^{13}C NMR spectrum (**Figure 44**) showed the corresponding olefinic carbon signals at δ 126.8 (C-4) and 129.7 (C-5), three oxygenated methine carbons at δ 68.6 (C-6), 70.8 (C-2) and 74.2 (C-3), as well as an oxygenated methylene signal at δ 66.7 (C-7).

In the HMBC spectrum (**Figure 48**), correlations of the proton signals of H-7a and H-7b with the carbonyl signal at δ 167.8 (C-7′), revealed that a benzoyloxy group was attached to C-7, while the correlation of H-3 signal with the C-7″ carbonyl signal at δ 167.1 indicated that the other benzoyloxy group was substituted at position 3.

To determine the relative configuration, the NOESY experiment (**Figure 49**) was performed. The 2D spectrum exhibited the correlation between H-7 and H-2, suggesting the relative conformation of these two positions. In addition, the coupling constant between the signals of H-2 and H-3 (J = 6.0 Hz) in the proton spectrum indicated the opposite arrangement of these protons.

Based on the spectral data analysis and comparison with reported values (Jolad *et al.*, 1981; Pan and Yu, 1995), compound EC-4 was identified as another cyclohexene derivative called zeylenol. Slight differences in the NMR assignments were confirmed in this study.

Zeylenol has been reported as a constituent of this plant by Kijjoa and his team in 2002, and also found in other annonaceous plants, all of which are from the genus *Uvaria* i.e. *Uvaria zeylanica* (Jolad *et al.*, 1982), *U. grandiflora* (Pan and Yu, 1995) and *U. kweichowensis* (Xu *et al.*, 2005). Moreover, it can be found in *Piper cubeb* (Taneja *et al.*, 1991) and *Kaempferia angustifolia* (Pancharoen *et al.*, 1989). Bioactivity evaluation of zeylenol showed antitumor activity against A549 bronchogenic carcinoma cell with IC_{50} value of 28 μg/ml, determined by MTT assay (Xu *et al.*, 2005).

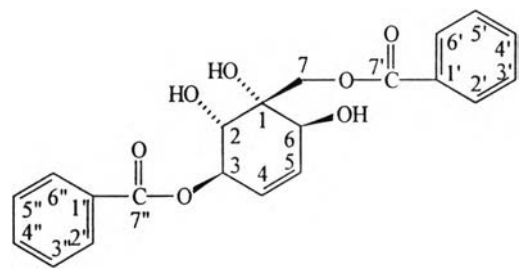

Table 20. Comparison of the ^{1}H and ^{13}C NMR spectral data of zeylenol and compound EC-4 (in $CDCl_3$, 500 MHz)

Position	Zeylenol		Compound EC-4	
	^{1}H†	^{13}C‡	^{1}H	^{13}C
1	-	76.0	-	75.9
2	4.32 (1H, *d*, *J* = 6.1 Hz)	68.7	4.22 (1H, *d*, *J* = 6.0 Hz)	70.8
3	5.70 (1H, *dddd*, *J* = 6.1, 2.6, 1.6, 1.1 Hz)	74.4	5.69 (1H, *m*)	74.2
4	5.88 (1H, *ddd*, *J* = 10.1, 2.6, 0.7 Hz)	127.0	5.84 (1H, *ddd*, *J* = 10.3, 2.5, 0.5 Hz)	126.8
5	5.99 (1H, *ddd*, *J* = 10.1, 4.0, 1.6 Hz)	129.5	5.98 (1H, *ddd*, *J* = 10.3, 4.0, 2.0 Hz)	129.7
6	4.32 (1H, *ddd*, *J* = 4.0, 1.1, 0.7 Hz)	70.9	4.32 (1H, *d*, *J* = 4.0 Hz)	68.6
7	4.75 (1H, *d*, *J* = 12.3 Hz)	66.8	4.72 (1H, *d*, *J* = 12.0 Hz)	66.7
	4.89 (1H, *d*, *J* = 12.3 Hz)		4.86 (1H, *d*, *J* = 12.0 Hz)	
1′	-	128.5	-	129.2
2′, 6′	7.3 – 8.1 (2H, *m*)	129.9	7.98 (2H, *dd*, *J* = 8.0, 1.5 Hz)	129.8
3′, 5′	7.3 – 8.1 (2H, *m*)	128.5	7.36 (2H, *m*)	128.4
4′	7.3 – 8.1 (1H, *m*)	133.5	7.51 (1H, *m*)	133.4
7′	-	165.0	-	167.8
1″	-	128.5	-	129.4
2″, 6″	7.3 – 8.1 (2H, *m*)	129.9	7.94 (2H, *dd*, *J* = 8.5, 1.5 Hz)	129.8
3″, 5″	7.3 – 8.1 (2H, *m*)	128.5	7.36 (2H, *m*)	128.4
4″	7.3 – 8.1 (1H, *m*)	133.5	7.51 (1H, *m*)	133.5
7″	-	165.0	-	167.1

† Pan and Yu, 1995 (in $CDCl_3$, 500 MHz)

‡ Jolad *et al.*, 1981 (in $CDCl_3$, 65 MHz)

1.5 Identification of Compound EC-5

Compound EC-5 was obtained as pale yellow powder, soluble in MeOH. The TOF mass spectrum (**Figure 52**) exhibited $[M+Na]^+$ ion peak at *m/z* 617, suggesting the molecular weight of 594 and the molecular formula $C_{27}H_{30}O_{15}$. The UV maxima at 208, 267 and 348 nm (**Figure 50**) and the presence in the ^1H NMR spectrum of an intramolecularly coupled hydroxy at the downfield chemical shift of δ 12.56 were indicative of a flavone skeleton (Markham, 1982; Robards and Antolovich, 1997). The IR spectrum (**Figure 51**) exhibited absorption bands for hydroxy and conjugated carbonyl functionalities at 3422 and 1659 cm^{-1}, respectively.

The ^1H NMR spectrum (**Figure 53a** and **53b**) exhibited two sets of *para*-substituted aromatic signals: the first ones at δ 7.98 (2H, *d*, 8.7 Hz, H-2′ and H-6′) and δ 6.85 (2H, *d*, 8.7 Hz, H-3′ and H-5′), the second ones at δ 7.35 (2H, *d*, 7.8Hz, H-2″ and H-6″) and 6.78 (2H, *d*, 7.8 Hz, H-3‴, H-5‴). The resonances of two *meta*-coupled aromatic protons could also be observed at δ 6.14 (1H, *d*, J = 1.8 Hz, H-6) and 6.37 (1H, *br s*, H-8). Two coupled olefinic proton signals at δ 7.34 (1H, *d*, J = 16.9 Hz, H-7‴) and 6.10 (1H, *d*, J = 16.9 Hz, H-8‴) suggested the presence of a *trans* double bond within the molecule. Furthermore, the presence of one anomeric proton signal at δ 5.44 (*d*, J = 6.6 Hz, H-1″) suggested that compound EC-5 should be a monoglycoside of kaempferol aglycone (Harborne, 1994).

The ^{13}C NMR (**Table 21** and **Figure 54**) and DEPT 135 spectra (**Figure 55**) displayed fifteen carbon signals of the kaempferol aglycone, together with six carbon signals, including an anomeric carbon resonance at δ 101.0, indicating a glucose unit within the molecule. The remaining carbon signals belong to a *p*-coumaric acid unit which includes a *trans* double bond, one carbonyl carbon and one aromatic ring *para*-substituted with a hydroxy group. Long-range HMBC correlation (**Figure 58**) between the carbonyl carbon (δ 166.0) of the *p*-coumaric acid unit and H-6″ methylene protons of the glucose unit confirmed the substitution of *p*-coumaric acid on C-6″ of the glucose molecule.

According to the above spectral evidence and by comparison of its NMR data with the reported data (Kumar *et al.*, 1985; Nikaido, Ohmoto and Sankawa, 1987), compound EC-5 was identified as tiliroside [kaempferol-3-*O*-(p-coumaroyl)-glucoside].

Tiliroside has been isolated from a number of plants from several families, e.g. *Rubus ulmifolius* (family Rosaceae) (Panizzi *et al.*, 2002), *Platanus acerifolia* (family Platanaceae) (Kaouadji, 1990) and *Siparuna apiosyce* (family Monimiaceae) (Leitao *et al.*, 2000). It was found to exhibit potent anti-complement activity in the bioassay-guided study of *Magnolia fargesii* (family Magnoliaceae) (Jung *et al.*, 1998) as well as the study of *Litsea japonica* (family Lauraceae) (Lee *et al.*, 2005), and also acted as an insect deterrent (Bajaj *et al.*, 1986). Moreover, the flavonoid was demonstrated to be a hepatoprotective principle from the flowers of *Tilia argentea* (family Tiliaceae) from its ability to inhibit SGPT and SGOT elevations (Matsuda *et al.*, 2002). It was also the most active flavonoid, isolated from *Helichrysum italicum,* displaying both *in vitro* antioxidant and *in vivo* anti-inflammatory activities (Sala *et al.*, 2003). It was slightly cytotoxic against a panel of human leukaemic cell lines (Dimas *et al.*, 2000), although its acetylated derivative, heptaacetyltiliroside, was more potent than the parent compound (Dimas *et al.*, 1999).

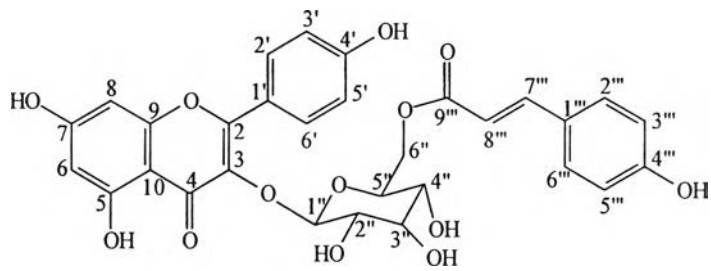

Table 21. Comparison of the ^{1}H and ^{13}C NMR spectral data of tiliroside and compound EC-5 (in DMSO-d_6, 300 MHz)

Position	Tiliroside†		Compound EC-5		
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC
2	-	156.4	-	156.2	
3	-	133.4	-	133.0	
4	-	177.5	-	177.2	
5	-	161.3	-	161.0	
6	6.19 (1H, *d*, *J* = 2.0 Hz)	98.5	6.14 (1H, *d*, *J* = 1.8 Hz)	98.8	C-8, C-10
7	-	164.2	-	164.2	
8	6.40 (1H, *d*, *J* = 2.0 Hz)	93.5	6.37 (1H, *br s*)	93.7	C-6, C-10
9	-	156.5	-	156.2	
10	-	103.7	-	103.8	
5-OH	12.60 (1H, *br s*)	-	12.56 (1H, *br s*)	-	
7-OH	10.13 (1H, *br s*)	-	10.12 (1H, *br s*)	-	
1′	-	120.7	-	120.7	
2′, 6′	8.01 (2H, *d*, *J* = 8.0 Hz)	130.0	7.98 (2H, *d*, *J* = 8.7 Hz)	130.7	C-2, C-2′, C-4′, C-6′
3′, 5′	6.89 (2H, *d*, *J* = 8.0 Hz)	115.7	6.85 (2H, *d*, *J* = 8.7 Hz)	115.1	C-1′, C-3′, C-5′
4′	-	159.7	-	159.8	
4′-OH	5.19 (*br s*)*	-	-	-	
1″	5.47 (1H, *d*, *J* = 7.0 Hz)	101.2	5.44 (1H, *d*, *J* = 6.6 Hz)	101.0	
2″	3.20 – 4.20 (4H, *m*)	74.3	3.16 – 3.47 (4H, *m*)	74.2	
3″		76.3		76.3	
4″		70.0		70.1	
5″		74.3		74.2	
6″	4.08 (1H, *dd*, *J* = 12.0, 2.0 Hz)	63.2	4.08 (1H, *dd*, *J* = 11.1, 5.7 Hz)	63.1	C-9‴
	4.35 (1H, *dd*, *J* = 12.0, 6.0 Hz)		4.27 (1H, *d*, *J* = 11.1 Hz)	-	
1‴	-	125.0	-	124.9	
2‴, 6‴	7.39 (2H, *d*, *J* = 8.0 Hz)	130.5	7.35 (2H, *d*, *J* = 7.8 Hz)	130.1	C-2‴, C-4‴, C-6‴, C-7‴
3‴, 5‴	6.81 (2H, *d*, *J* = 8.0 Hz)	115.2	6.78 (2H, *d*, *J* = 7.8 Hz)	115.7	C-1‴, C-3‴, C-5‴
4‴	-	159.7	-	159.6	
4‴-OH	5.45 (*br s*)*	-	-	-	
7‴	7.37 (1H, *d*, *J* = 16.0 Hz)	144.2	7.34 (1H, *d*, *J* = 16.9 Hz)	144.5	C-2‴, C-6‴, C-9‴
8‴	6.12 (1H, *d*, *J* = 16.0 Hz)	113.7	6.10 (1H, *d*, *J* = 16.9 Hz)	113.6	C-1‴
9‴	-	165.8	-	166.0	-

† Kumar *et al.*, 1985 (in DMSO-d_6, 100 MHz)

* may be exchangeable

1.6 Identification of Compound EC-6

Compound EC-6 was obtained as pale yellow amorphous powder, soluble in MeOH. Its TOF mass spectrum (**Figure 61**) exhibited $[M+Na]^+$ ion peak at *m/z* 617, suggesting the molecular weight of 594 and the molecular formula $C_{27}H_{30}O_{15}$. The UV absorption maxima at 207, 267 and 313 nm (**Figure 59**) and the presence of a chelated hydroxy singlet in the ^1H NMR spectrum at δ 12.54 were indicative of a flavone skeleton (Markham, 1982; Robards and Antolovich, 1997). The IR absorption bands (**Figure 60**) at 3460 and 1684 cm^{-1} confirmed the presence of hydroxy and conjugated carbonyl functionalities, respectively.

The ^1H NMR spectrum (**Figure 62**) exhibited the *ortho*-coupled signals at δ 7.97 (2H, *d*, *J* = 8.4 Hz, H-2′ and H-6′) and δ 6.86 (2H, *d*, *J* = 8.4 Hz, H-3' and H-5′), indicating *para*-substitution for ring B of the flavone nucleus. In addition, the presence of two anomeric proton signals at δ 5.29 (1H, *d*, *J* = 7.2, H-1″) and 5.09 (1H, *br s*, H-1‴) suggested that compound EC-6 should be diglycoside of kaempferol (Harborne, 1994). One of the sugar should be rhamnose, judging from the methyl doublet (*J* = 6.0 Hz) of H-6‴ observed at δ 0.97.

The ^{13}C NMR (**Table 22** and **Figure 63**) and HMQC spectra (**Figure 66**) displayed twelve signals of two sugar units, including two anomeric carbons at δ 100.8 (C-1″) and 101.4 (C-1‴), in addition to fifteen carbon signals of the kaempferol nucleus.

The three-bond correlation, observed in the HMBC spectrum (**Figure 67**), of the anomeric H-1″ with C-3 (δ 133.2) indicated that the glucose unit was connected through an oxygen atom to the kaempferol aglycone at C-3 (*O*-glycoside). On the other hand, the correlations of H-1‴ with C-3‴ (δ 70.5) and of H-1‴ with C-6″ (δ 67.0) indicated that rhamnose, as the second sugar unit, was connected to glucose with a 1,6-linkage. This disaccharide is called rutinoside.

In conclusion, compound EC-6 was elucidated as kaempferol-3-*O*-rutinoside based on spectral data analysis and comparison with previously reported values (Ho *et al.*, 2002). Kaempferol-3-*O*-rutinoside was commonly found in various plants e.g. *Hedyotis herbacea* (Hamzah and Lajis, 1998), *Hypericum perforatum* (Silva *et al.*, 2005), *Warburgia ugandensis* (Manguro *et al.*, 2003), *Morinda morindoides* (Cimanga *et al.*, 1995) and *Alternanthera brasiliana* (Brochado *et al.*, 2003). It has been found to possess radical scavenging activity (Hou *et al.*, 2005), but caused little

or no effect on the viability of Hep G2 cell in the study of cytotoxic flavonoid from the flowers of *Daphnis genkwae* (Lin *et al.*, 2001).

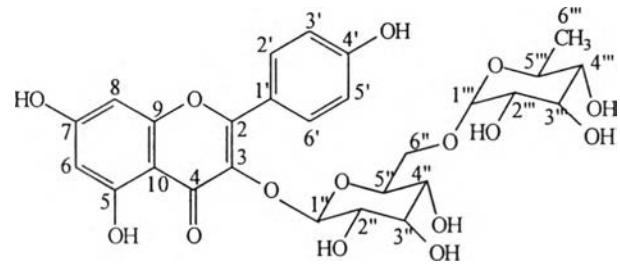

Table 22. Comparison of the NMR spectral data of daempferol-3-*O*-rutinoside and compound EC-6 (in DMSO-d_6, 300 MHz)

Position	Kaempferol-3-*O*-rutinoside†		Compound EC-6		
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC
2	-	156.3	-	156.4	
3	-	133.0	-	133.2	
4	-	177.1	-	177.2	
5	-	161.0	-	161.1	
6	6.20 (1H, *d*, *J* = 2.1 Hz)	98.6	6.18 (1H, *br s*)	98.9	C-7, C-8, C-10
7	-	163.9	-	164.3	
8	6.40 (1H, *d*, *J* = 2.1 Hz)	93.6	6.39 (1H, *br s*)	93.9	C-6, C-7, C-9, C-10
9	-	156.7	-	156.7	
10	-	103.9	-	103.9	
5-OH	12.55	-	12.54	-	
7-OH	10.83	-	-	-	
1′	-	120.7	-	120.9	
2′, 6′	8.00 (2H, *d*, *J* = 9.6 Hz)	130.7	7.97 (2H, *d*, *J* = 8.4 Hz)	130.8	C-1′, C-2′, C-4′, C-6′
3′, 5′	6.87 (2H, *d*, *J* = 9.6 Hz)	114.9	6.86 (2H, *d*, *J* = 8.4 Hz)	115.1	C-3′, C-5′
4′	-	159.7	-	159.8	
1″	5.32 (1H, *d*, *J* = 7.0 Hz)	101.2	5.29 (1H, *d*, *J* = 7.2 Hz)	100.8	C-3
2″	3.15-3.69 (5H, *m*)	74.1	3.03-3.69 (5H, *m*)	74.3	
3″		76.3		76.5	
4″		69.9		70.7	
5″		75.7		75.9	
6″		66.8		67.0	
1‴	5.07 (1H, *d*, *J* = 8.0 Hz)	100.7	5.09 (1H, *br s*)	101.4	C-6″, C-3‴
2‴	3.42-4.08 (4H, *m*)	70.3	3.03-3.69 (4H, *m*)	70.1	
3‴		70.5		70.5	
4‴		71.7		71.9	
5‴		68.2		68.4	
6‴	0.98 (1H, *d*, *J* = 6.3 Hz)	17.8	0.97 (1H, *d*, *J* = 6.0 Hz)	18.0	C-5‴, C-4‴

† Ho *et al.*, 2002 (in DMSO-d_6)

1.7 Identification of Compound EC-7

Compound EC-7 was obtained as orange amorphous solid. Its UV spectrum (**Figure 68**) showed characteristics of a phenanthrene chromophore at λ_{max} 246, 263, 273 and 438 nm. The molecular formula was determined as $C_{18}H_{11}NO_4$ by ESITOF mass spectrometry (**Figure 70**), observing $[M+H]^+$ at *m/z* 306. The IR spectrum (**Figure 69**) of this compound revealed the presence of a carbonyl group at 1712 cm^{-1}.

The ^1H NMR spectrum (**Figure 71**) displayed one methoxy signal at δ 3.97 (3H, *s*), one methylenedioxy signal at δ 6.33 (2H, *s*) and six aromatic protons at δ 7.12 (1H, *s*), 7.28 (1H, *d*, *J* = 9.3 Hz), 7.75 (1H, *d*, *J* = 5.1 Hz), 7.98 (1H, *s*), 8.54 (1H, *d*, *J* = 9.3 Hz) and 8.86 (1H, *d*, *J* = 5.1 Hz). The ^{13}C NMR spectrum (**Figure 72**) exhibited, in total, eighteen carbon signals which could be classified as those of one methoxy carbon at δ 55.8, one methylenedioxy carbon at δ 102.3, fifteen aromatic carbons, and the most downfield quaternary signal at δ 182.3 assignable to carbonyl moiety at C-7, suggesting the oxoaporphine-type alkaloid.

As can be determined from the coupling constants, compound EC-7 possesses two systems of coupled aromatic protons. One of them is the doublet at δ 7.75, coupling with the signal at δ 8.86 with the coupling constant of 5.1, assignable to H-4 and H-5, respectively. The other system is the doublet at δ 7.28, which coupled with another doublet at δ 8.54 with ortho-coupling constant of 9.3, attributed to two ortho-protons on the aromatic ring D of the aporphine skeleton. These couplings were also supported by ^1H-^1H COSY spectrum (**Figure 74**). The splitting pattern of these protons and the remaining aromatic protons indicated that the methoxy group should be located either at C-9 or C-10 of ring D, and the methylenedioxy group should be placed on ring A.

Compound EC-7 was eventually identified as the alkaloid lanuginosine by the analysis of all spectral data and comparison to earlier reports (Wijeratne *et al.*, 1996; Zhang *et al.*, 2002).

Lanuginosine is an oxoaporphine alkaloid previously found in the bark of two plants of the family Magnoliaceae: *Michelia lanuginosa* (Talapatra, Patra and Talapatra, 1975) and *Magnolia obovata* (Pyo, Yun-Choi and Hong, 2003). It was also reported as a constituent of some annonaceous species such as *Duguetia glabriuscula* (Sequeira *et al.*, 2001) and *Enantia pilosa* (Nieto, Cave and Leboeuf, 1976).

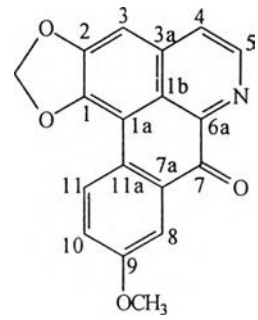

Table 23. Comparison of the ^{1}H and ^{13}C NMR spectral data of lanuginosine and compound EC-7 (in $CDCl_3$, 300 MHz)

Position	Lanuginosine		Compound EC-7	
	^{1}H†	^{13}C‡	^{1}H	^{13}C
1	-	147.8	-	148.0
1a	-	108.7	-	108.2
1b	-	123.2	-	122.7
2	-	152.2	-	151.8
3	7.08 (1H, *s*)	102.9	7.12 (1H, *s*)	102.4
3a	-	145.8	-	145.3
4	7.73 (1H, *d*, *J* = 5.2 Hz)	124.6	7.75 (1H, *d*, *J* = 5.1 Hz)	124.3
5	8.86 (1H, *d*, *J* = 5.2 Hz)	145.3	8.86 (1H, *d*, *J* = 5.1 Hz)	144.8
6a	-	136.2	-	135.9
7	-	183.0	-	182.3
7a	-	126.6	-	124.3
8	7.98 (1H, *d*, *J* = 2.4 Hz)	110.8	7.98 (1H, *s*)	110.3
9	-	160.2	-	159.8
10	7.24 (1H, *dd*, *J* = 8.9, 2.2 Hz)	123.0	7.28 (1H, *d*, *J* = 9.3 Hz)	122.6
11	8.48 (1H, *d*, *J* = 8.9 Hz)	129.5	8.54 (1H, *d*, *J* = 9.3 Hz)	129.1
11a	-	133.3	-	132.9
$-OCH_2O-$	6.33 (2H, *s*)	102.7	6.33 (2H, *s*)	102.3
$-OCH_3$	3.99 (3H, *s*)	56.2	3.97 (3H, *s*)	55.8

† Wijeratne *et al.*, 1996

‡ Zhang *et al.*, 2002 (in $CDCl_3$, 125 MHz)

2. Structure Determination of Compounds Isolated from *Stelechocarpus cauliflorus*

2.1 Identification of Compound SC-1

Compound SC-1 was obtained as white amorphous powder. Its molecular formula, $C_{22}H_{28}O_5$ was determined from the $[M^+]$ ion peak at *m/z* 372 (**Figure 78**). The UV absorption maxima (**Figure 76**) at 232, 243, 258 and 281 nm and the IR bands at 1517 and 1462 cm^{-1} (**Figure 77**) indicated the presence of aromatic ring in the molecule.

The ^1H NMR spectrum (**Figure 79**) exhibited one methyl signal at δ 1.04 (*d*, 6.5), two methine signals at δ 4.52 (*d*, *J* = 6.5 Hz, H-7/H-7′) and 2.33 (*m*, H-8/H-8′), three aromatic proton signals at δ 6.99 (*d*, *J* = 2.0 Hz, H-2/H-2′), 6.97 (*dd*, *J* = 8.3, 2.0 Hz, H-6/H-6′) and 6.85 (*d*, *J* = 8.3 Hz, H-5/H-5′), and two methoxy signals at δ 55.9 and 55.8.

The methyl doublet at δ 1.04 was coupled to the methine resonance at δ 4.52, which, in turn, was also coupled with another methine signal at δ 2.33, as shown in the ^1H-^1H COSY spectrum (**Figure 82**). One aromatic proton doublet at δ 6.85 was *ortho*-coupled (*J* = 8.3 Hz) with another aromatic proton which resonated at δ 6.97. The latter proton was also *meta*-coupled (*J* = 2.0 Hz) to a doublet at δ 6.99. This pattern of coupled aromatic protons indicated substitution of two methoxy groups on the aromatic ring.

Long-range HMBC correlations (**Figure 84**) from H-7 signal at δ 4.52 to both C-2 (δ 109.7) and C-6 (δ 118.6), and from H-2 signal at δ 6.99 to C-7 (δ 67.2) established the connectivity between the aromatic ring at position 1 and the C_3 unit at position 7. The symmetry between both sides of the molecule was confirmed by the HMBC correlation between H-8 signal at δ 2.33 to C-8′ (δ 44.4), and *vice versa*.

Through analysis of the above spectral data and comparison with literature (Holloway and Scheinmann, 1974; Kuo *et al.*, 2002), compound SC-1 was therefore identified as the 2,5 diaryl-3,4-dimethyltetrahydrofuran lignan named galgravin.

This lignan, isolated from *Piper kadsura,* effectively suppressed hepatitis B virus surface antigen production at the concentration of 25 μM (Huang *et al.*, 2001). The compound also acted as an immunosuppressive agent by potently inhibiting human mononuclear cell proliferation and interferon production (Kuo *et al.*, 2002). Galgravin, isolated from *Aristolochia arcuta,* showed neuroprotective effect by

promoting neuronal survival and neurite outgrowth (Zhai *et al.*, 2005). This lignan, isolated from *Nectandra megapotamica* (Filho *et al.*, 2004) and *Piper futokadsura* (Chen, Yu and Xu, 1993), respectively, displayed anti-inflammatory and anti-platelet activating factor (PAF) activities.

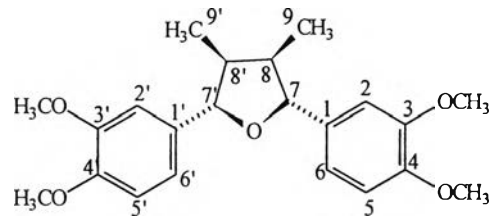

Table 24. Comparison of the ^{1}H and ^{13}C NMR spectral data of galgravin and compound SC-1 (in $CDCl_3$, 500 MHz)

Position	Galgravin		Compound SC-1		HMBC
	^{1}H†	^{13}C‡	^{1}H	^{13}C	
1	-	134.8	-	134.8	
2	7.00 (1H, *m*)	109.7	6.99 (1H, *d*, *J* = 2.0 Hz)	109.7	C-4, C-6, C-7
3	-	148.9	-	148.9	
4	-	148.4	-	148.8	
5	7.00 (1H, *m*)	110.9	6.85 (1H, *d*, *J* = 8.3 Hz)	110.9	C-1, C-3
6	7.00 (1H, *m*)	118.5	6.97 (1H, *dd*, *J* = 8.3, 2.0 Hz)	118.6	C-2
7	4.53 (1H, *m*)	87.2	4.52 (1H, *d*, *J* = 6.5 Hz)	87.2	C-2, C-6, C-8, C-9
8	2.40 (1H, *m*)	44.3	2.33 (1H, *m*)	44.4	C-8′
9	1.05 (3H, *d*)	12.9	1.04 (3H, *d*, *J* = 6.5 Hz)	12.9	C-7, C-8
1′	-	134.8	-	134.8	
2′	7.00 (1H, *m*)	109.7	6.99 (1H, *d*, *J* = 2.0 Hz)	109.7	C-4′, C-6′, C-7′
3′	-	148.9	-	148.9	
4′	-	148.4	-	148.8	
5′	7.00 (1H, *m*)	110.9	6.85 (1H, *d*, *J* = 8.3 Hz)	110.9	C-1′, C-3′
6′	7.00 (1H, *m*)	118.5	6.97 (1H, *dd*, *J* = 8.3, 2.0 Hz)	118.6	C-2’
7′	4.53 (1H, *m*)	87.2	4.52 (1H, *d*, *J* = 6.5 Hz)	87.2	C-2′,C-6′, C-8’,C-9′
8′	2.40 (1H, *m*)	44.3	2.33 (1H, *m*)	44.4	C-8
9′	1.05 (3H, *d*)	12.9	1.04 (3H, *d*, *J* = 6.5 Hz)	12.9	C-7′, C-8′
3-OCH_3	3.87 (3H, *s*)	55.8	3.88 (3H, *s*)	55.8	
4-OCH_3	3.87 (3H, *s*)	55.9	3.88 (3H, *s*)	55.9	
3′-OCH_3	3.87 (3H, *s*)	55.8	3.88 (3H, *s*)	55.8	
4′-OCH_3	3.87 (3H, *s*)	55.9	3.88 (3H, *s*)	55.9	

† Holloway and Scheinmann, 1974 (in $CDCl_3$, 60 MHz)

‡ Kuo *et al.*, 2002 (in $CDCl_3$, 75 MHz)

2.2 Identification of Compound SC-2

Compound SC-2, a light brown oil, exhibited a $[M+Na]^+$ peak at *m/z* 243 in the ESITOF mass spectrum (**Figure 87**), indicating a molecular formula of $C_{15}H_{24}O$. The IR spectrum of compound SC-2 (**Figure 86**) showed the absorption peaks of hydroxy function and double bond at 3375 and 1462 cm^{-1}, respectively, while its UV exhibited absorption maxima at 232 and 245 nm (**Figure 85**).

The ^{13}C-NMR spectrum (**Figure 89**) exhibited 15 carbon peaks classified into those of three methyl (δ 16.3, 26.1 and 27.4), five methylene (δ 24.8, 26.6, 38.8, 41.7 and 106.2), four methine (δ 26.7, 29.9, 53.4 and 54.3), three quaternary carbons (δ 20.2, 80.9 and 153.4) by the DEPT 135 experiment (**Figure 90**).

The proton NMR (**Figure 88**) exhibited three methyl singlets at δ 1.01, 1.03 and 1.26, which correlated to the carbon signals at 27.4, 16.3 and 26.1, respectively. The ^{1}H-NMR spectrum also showed an exomethylene signal at δ 4.65 (2H, *d*, J = 7.8 Hz), corresponded to the ^{13}C-NMR spectrum which exhibited the signals due to olefinic carbons at δ 106.2 and 153.4.

Compound SC-2 was identified as spathulenol, an aromadendrane sesquiterpenoid, by analysis of the above spectral data and confirmed by comparison with previously published data (Iwabuchi, Yoshikura and Kamisako, 1989).

A large number of plants in the family Annonaceae are fragrant due to the presence of essential oils in these plants. Major components of these oils are monoterpenes and sesquiterpenes. Spathulenol is a common sesquiterpenes found widely in plants such as *Panax ginseng* (Iwabuchi *et al.*, 1989), *Nepeta depauperata* (Mehrabani, Asadipour and Amoli, 2004) and *Salvia vermifolia* (Nacar and Ilcim, 2002). Examples of annonaceous plants reported as possessing spathulenol are *Xylopia aromatica* (Fournier *et al.*, 1994), *Monanthotaxis diclina* and *Unonopsis guatterioides* (Fournier *et al.*, 1999) and *Guatteria* sp. (Maia *et al.*, 2005). Spathulenol, isolated from *Xylopia brasiliensis,* was reported as exhibiting antifungal activity (Moreira *et al.*, 2003).

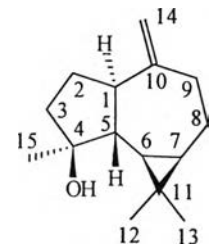

Table 25. Comparison of the ^{1}H and ^{13}C NMR spectral data of spathunol and compound SC-2 (in $CDCl_3$, 300 MHz)

Position	Spathulenol†		SC-2	
	^{1}H	^{13}C	^{1}H	^{13}C
1		54.5	2.17 (1H, *m*)	54.3
2a		26.8	1.47 (1H, *m*)	26.6
2b			1.79 (1H, *m*)	
3a		41.8	1.61 (1H, *m*)	41.7
3b			1.73 (1H, *m*)	
4		80.9	-	80.9
5		53.4	1.29 (1H, *m*)	53.4
6	0.47 (1H, *dd*, *J* = 11.5, 9.5 Hz)	30.0	0.45 (1H, *t*, *J* = 10.2 Hz)	29.9
7	0.71 (1H, *ddd*, *J* = 11.4, 9.4, 5.9 Hz)	27.7	0.69 (1H, *dd*, *J* = 10.2, 6.9 Hz)	26.7
8a		24.9	0.94 (1H, *m*)	24.8
8b			2.02 (1H, *m*)	
9a		39.0	2.10 (1H, *m*)	38.8
9b	2.42 (1H, *m*)		2.40 (1H, *m*)	
10		153.5	-	153.4
11		20.3	-	20.2
12	1.04 (3H, *s*)	28.7	1.01 (3H, *s*)	27.4
13	1.06 (3H, *s*)	16.4	1.03 (3H, *s*)	16.3
14	4.67, 4.69 (2H, *br* s)	106.3	4.65 (2H, *d*, *J* = 7.8 Hz)	106.2
15	1.28 (3H, *s*)	26.1	1.26 (3H, *s*)	26.1

† Iwabuchi *et al.*, 1989 (in $CDCl_3$, 400 MHz)

2.3 Identification of Compound SC-3

Compound SC-3 was obtained as a white needle crystal, soluble in $CHCl_3$. The ESITOF mass spectrometry (**Figure 93**) exhibited the molecular ion at *m/z* 326, corresponding to the molecular formular $C_{20}H_{22}O_4$. The UV absorptions at 234, 244, 250 and 279 nm (**Figure 91**) and the IR bands (**Figure 92**) for hydroxyl group (3456 cm^{-1}), aromatic and olefinic moeity (1606 and 1517 cm^{-1}) were suggestive of a hydroxy substituted aromatic ring and olefinic moeity in the molecule.

The ^1H NMR signals (**Table 26** and **Figure 94**) displayed two methyl, two methoxyl, five aromatic and four methine protons. According to the coupling constant, the methine protons are coupling in two pairs, which are the couple between signal at δ 6.09 (1H, *dq*, 15.86, 6.71) and 6.35 (1H, *dd*, 15.86, 1.53) and the couple between δ 5.08 (1H, *d*, 9.46) and 3.43 (1H, *dq*, 9.46, 6.71) with coupling constant 15.86 and 9.46, respectively. Two olefinic methine protons of the first pair are coupling with coupling constant 15.86 indicated trans conformation. This result was confirmed with the ^1H-^1H COSY spectrum (**Figure 97**) which also exhibited further correlation to both two pairs, which are the correlation between the signal at δ 3.43 and the methyl signal at δ 1.36 (3H, *d*, 6.71) and the correlation between signal at δ 6.09 and 1.85 (3H, *d*, 6.71). The latter correlation contained two olefinic methine protons connected to methyl group are called propenyl group.

The ^{13}C NMR (**Table 26** and **Figure 95**) and DEPT 135 (**Figure 96**) spectra showed twenty carbon signals corresponding to two methyls, two methoxyls, nine methines and eight quaternary carbons. The HMBC spectra (**Figure 99a** and **99b**) revealed the correlation between H-6 (δ 6.77) and C-8 (δ 131.0), indicated that propenyl group should placed at C-5 of aromatic ring. The correlation between H-2 (δ 5.08) and C-2′ (δ 109.0) and 6’ (δ 120.0) on the other aromatic ring indicated that substituted aromatic ring attached to C-3 of the molecule. The molecule contained dimer of C_6-C_3 which connected to each other at position 3a and 7a are benzofuran type of neolignan. The methoxy groups substitutions at C-3′ and C-7 were confirmed by HMBC experiment.

The relative configuration of two chiral carbons at C-2 (δ 93.7) and C-3 (δ 75.6) were measured by NOESY experiment. The NOESY spectrum (**Figure 100**) displayed the correlation between H-2 and methyl protons at C-3 indicated that those

protons are in the same side and aromatic protons attachted to C-2 also correlated to proton at H-3 which indicated the same side to H-3 and the opposited side to H-2.

From the above ^{1}H and ^{13}C NMR data, together with the information from ^{1}H-^{1}H COSY, HMQC (**Figure 98**) and HMBC experiments, compound SC-3 was identified as a 2,3 dihydrobenzofuran neolignan, licarin A which was reported in previous research (Achenbach *et al.*, 1987). Licarin A is an optically active form of dehydrodiisoeugenol (Quesne *et al.*, 1980).

This compound has previously been isolated from several plants of the family Lauraceae e.g. from the leaves of *Machilus japonica* (Gonzalez-Coloma *et al.*, 1994), *Machilus thunbergii* (Shimomura *et al.*, 1987), wood of *Licaria aritu* wood (Aiba *et al.*, 1973), fruits of *Nectandra glabrescens* (Barbosa-Filho *et al.*, 1989) and the branch wood of *Urbanodendrom verrucosum* (Dias *et al.*, 1982). In addition, it was found in the leaves of *Magnolia kachirachirai*, family Magnoliaceae (Ito *et al.*, 1984).

Licarin A was shown to induce apoptotic effect in human promyeloid leukemic HL-60 cells by the activation of caspase-3. This lignan therefore possesses potential to act as cancer chemopreventive agent (Saleem *et al.*, 2005). It also displayed cytotoxic activity against KB cell line with ED_{50} value of 7.0 μg/ml (Quesne *et al.*, 1980). Additionally, licarin A, in the form of dehydrodiisoeugenol from mace of *Myristica fragrans,* completely inhibited the growth of *Streptococcus mutans* at a concentration of 12.5 μg/ml (Hattori *et al.*, 1986).

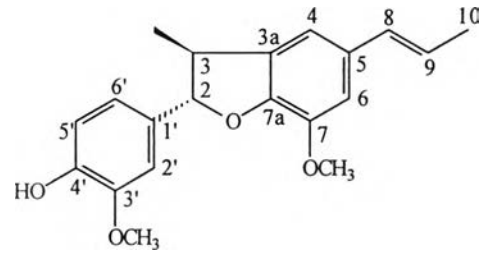

Table 26. Comparison of the ^{1}H and ^{13}C NMR spectral data of licarin A and compound SC-3 (in $CDCl_3$, 300 MHz)

Position	Licarin A†		Compound SC-3	
	^{1}H†	^{13}C‡	^{1}H	^{13}C
2	5.05 (1H, *d*, 9.30)	94.0	5.08 (1H, *d*, 9.46)	93.7
3	3.38 (1H, *m*)	46.3	3.43 (1H, *dq*, 9.46, 6.71)	45.6
3a	-	134.6	-	133.3
4	6.77–7.07 (5H, *m*)	114.4	6.75 (1H, *br s*)	113.3
5		133.0	-	132.2
6	6.77–7.07 (5H, *m*)	111.4	6.77 (1H, *br s*)	109.2
7	-	145.1	-	144.1
7a	-	147.9	-	146.7
8	6.31 (1H, *d*, 16.0)	132.1	6.35 (1H, *dd*, 15.86, 1.53)	131.0
9	5.89-6.26 (1H, *m*)	123.3	6.09 (1H, *dq*, 15.86, 6.71)	123.5
10	1.81 (3H, *d*, 5.10)	18.5	1.85 (3H, *d*, 6.71)	18.4
1′	-	132.9	-	132.1
2′	6.77–7.07 (5H, *m*)	110.8	6.96 (1H, *d*, 1.22)	109.0
3′	-	148.4	-	146.7
4′	-	147.6	-	145.8
5′	6.77–7.07 (5H, *m*)	115.7	6.78 (1H, *d*, 7.93)	114.1
6′	6.77–7.07 (5H, *m*)	120.3	6.89 (1H, *dd*, 8.24, 1.53)	120.0
3-CH_3	1.33 (3H, *d*, 6.80)	18.0	1.36 (3H, *d*, 6.71)	17.6
7-OCH_3	3.81 (3H, *s*)	56.4,	3.85 (3H, *s*), 3.88 (3H, *s*)	56.0
3′-OCH_3	3.81 (3H, *s*)	56.5	3.85 (3H, *s*), 3.88 (3H, *s*)	56.0
4′-OH	7.56 (1H, *s*)	-	5.66 (1H, *s*)	-

† Achenbach *et al.*, 1987 (in $CDCl_3$, 400 MHz)

2.4 Identification of Compound SC-4

Compound SC-4 was obtained as a yellow oil. The UV absorption maxima (**Figure 101**) at 233, 244, 254 and 280 nm and the IR peaks at 1600 and 1517 cm^{-1} suggested the presence of aromatic and olefinic moieties within the structure. Its ESITOF mass spectrum (**Figure 103**) exhibited the molecular ion peak at *m/z* 340, corresponding to the molecular formula $C_{21}H_{24}O_4$. The difference in molecular weight of this compound from that of compound SC-3 indicated a methoxy group in place of a hydroxy group in the latter compound.

The ^1H and ^{13}C NMR spectra (**Figure 104** and **105**) of compound SC-4 are very similar to compound SC-3 except for one additional methoxy signal and a slight difference in the coupling pattern of aromatic protons. ^1H-^1H COSY spectrum (**Figure 107**) exhibited three set of correlated signals. The first was among the signals at δ 5.09 (1H, *d*, *J* = 9.5 Hz), 3.44 (1H, *dq*, *J* = 9.5, 6.7 Hz) and 1.36 (1H, *d*, *J* = 7.0 Hz) which were assigned to H-2, H-3 and methyl protons at C-3, respectively. The second set was the signals at 6.34 (1H, *dd*, *J* = 15.6, 1.7 Hz), 6.09 (1H, *dq*, *J* = 15.6, 6.7 Hz) and 1.84 (1H, *dd*, *J* = 6.7, 1.7 Hz) of the *trans*-propenyl group. This group was located at C-5, as confirmed by the HMBC correlations (**Figure 109a**) of H-8 (δ 6.34) to both C-4 (δ 113.2) and C-6 (δ 109.1) and between H-9 (δ 6.09) and C-5 (δ 132.1). The last one was the correlation between H-2′ (δ 6.97, *d*, *J* = 2.0 Hz) and H-6′ (δ 6.93, *dd*, *J* = 8.1, 2.0 Hz), which, in turn, coupled to H-5′ and C-2 indicated the dimethoxyl substituted aromatic ring placed to C-2. Position H-5′ in SC-3 was downfield than that of compound SC-4 because of the effect of hydroxyl group substitution at C-4′. All methine carbons were assigned based on information of HMQC spectrum (**Figure 108a** and **108b**).

From the NOESY spectrum (**Figure 110**), H-3 showed correlations to both aromatic H-2′ and H-6′, whereas H-2 showed cross peak to the methyl group at C-3, indicating that H-3 and the aromatic ring at C-2 were located on the same side of the molecule, while H-2 and methyl group at C-3 were in the opposite direction.

From all of the above spectroscopic data in comparison with reported values (El-Feraly *et al.*, 1982), compound SC-4 was identified as a 2,3-dihydrobenzofuran neolignan called acuminatin. Its negative optical rotation indicated it to be (-)-acuminatin. (+)-Acuminatin was isolated from the trunk of *Aniba* species (Fernandes *et al.*, 1976) and *Nectandra miranda* (Aiba *et al.*, 1977), whereas (-)-acuminatin was

found in *Piper kadsura* (Ma, Han and Wang, 1993). Both optical isomers of acuminatin were found in the same plant, that is, *Magnolia kachirachirai* (El-Feraly *et al.*, 1982; Ito *et al.*, 1984). (-)-Acuminatin exhibited weak cytotoxicity against colon carcinoma (HT-29) and human breast carcinoma (MCF-7) cell lines (Li *et al.*, 2004).

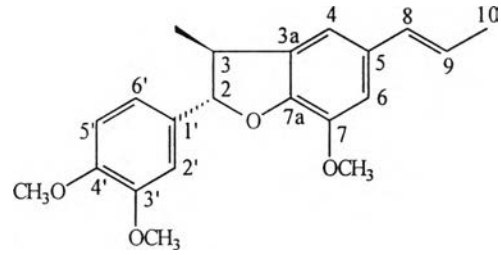

Table 27. Comparison of the ^{1}H and ^{13}C NMR spectral data of acuminatin and compound SC-4 (in $CDCl_3$, 500 MHz)

Position	Acuminatin		SC-4	
	^{1}H†	^{13}C‡	^{1}H	^{13}C
2	5.01 (1H, *d*, *J* = 9.0 Hz)	93.6	5.09 (1H, *d*, *J* = 9.5 Hz)	93.6
3	3.0-3.7 (1H, *m*)	45.7	3.44 (1H, *dq*, *J* = 9.5, 6.7 Hz)	45.5
3a	-	133.1	-	133.2
4	6.6-7.0 (1H, *m*)	113.6	6.75 (1H, *br s*)	113.2
5		135.5	-	132.1
6	6.6-7.0 (1H, *m*)	110.1	6.77 (1H, *br s*)	109.1
7		144.3	-	144.1
7a		146.9	-	146.5
8	6.4-6.6 (1H, *m*)	131.2	6.34 (1H, *dd*, *J* = 15.6, 1.7 Hz)	130.8
9	5.8-6.2 (1H, *m*)	123.3	6.09 (1H, *dq*, *J* = 15.6, 6.7 Hz)	123.4
10	1.85 (3H, *d*, *J* = 5.0 Hz)	18.3	1.84 (3H, *dd*, *J* = 6.7, 1.7 Hz)	18.3
1′		132.4	-	132.6
2′	6.6-7.0 (1H, *m*)	110.0	6.97 (1H, *d*, *J* = 2.0 Hz)	109.2
3′		149.5	-	149.0
4′		149.5	-	149.0
5′	6.6-7.0 (1H, *m*)	111.5	6.82 (1H, *d*, *J* = 8.1 hz)	110.7
6′	6.6-7.0 (1H, *m*)	119.2	6.93 (1H, *dd*, *J* = 8.1, 2.0 Hz)	119.1
3-CH_3	1.36 (3H, *d*, *J* = 7.0 Hz)	17.8	1.36 (3H, *d*, *J* = 7.0 Hz)	17.5
7-OCH_3	3.81 (3H, *s*)	56.0, 56.1	3.85 (3H, *s*)	55.8
3′-OCH_3	3.85 (3H, *s*)		3.85 (3H, *s*)	55.8
4′-OCH_3			3.87 (3H, *s*)	55.9

† Fernandes *et al.*, 1976

‡ El-Feraly *et al.*, 1982 (in $CDCl_3$, 15.03 MHz)

2.5 Identification of Compound SC-5

Compound SC-5 was obtained as light brown amorphous powder, soluble in MeOH. Its EI mass spectrum (**Figure 113**) exhibited the molecular ion peak at *m/z* 434, in agreement with $C_{21}H_{22}O_{10}$ as the molecular formula. Two major mass fragments were observed at *m/z* 434 and 270, the latter resulted from losing one hexose sugar unit. The UV absorption spectrum (**Figure 111**) showed λ_{max} at 235, 293 and 335 nm. The IR spectrum (**Figure 112**) showed bands at 3413 (O-H stretching), 1643 (C=O stretching) and 1469 (aromatic ring) cm^{-1}.

The ^{1}H NMR spectrum (**Figure 114**) displayed four aromatic proton signals integrated for six protons, five methine protons connected to heteroatom (resonated at δ 4.25 – 3.31) and one methyl doublet (δ 1.18, *J* = 6.5 Hz) assignable to H-6″ of the rhamnose sugar unit within the molecule.

The ^{13}C NMR data of compound SC-5 (**Figure 115**) exhibited typical signals of a flavanonol skeleton (**Table 28**): chemical shifts of C-2 at δ 83.9 and C-3 at δ 78.7. The single bond between C-2 and C-3 was confirmed by the presence of two doublets (*J* = 10.8 Hz) at δ 4.62 (H-3) and 5.14 (H-2) in the ^{1}H NMR spectrum (**Figure 114**). Thus, the aglycone moiety was identified as the known dihydrokaempferol attached to a rhamnose unit at C-3. The HMBC cross peaks (**Figure 119**) between H-3 (δ 4.62, *d*, *J* = 10.8 Hz) and C-1″ (δ 102.2), and between H-1″ (δ 4.01, *d*, *J* = 1.5 Hz) and C-3 (δ 78.7), confirmed the glycosylation at this position.

The OH group attached to C-3 was assigned the *trans* configuration in relation to the B-ring according to a large coupling constant (*J* = 10.8 Hz) between H-2 and H-3. This was supported by 2D-NOESY spectrum (**Figure 120**), which showed correlations between H-3 and H-1″, H-3″ and H-5″. In the aromatic proton region, a set of *meta*-coupled signals at δ 5.89 (1H, *d*, *J* = 2.3 Hz, H-6) and 5.92 (1H, *d*, *J* = 2.3 Hz, H-8), and another set of *ortho*-coupled resonances at δ 7.35 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′) and 6.84 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), could be observed. All methine and quaternary carbons were assigned by ^{1}H-^{1}H COSY (**Figure 117**), HMQC (**Figure 118**) and HMBC spectra (**Figure 119**).

Compound SC-5 was identified as engeletin (dihydroflavonol-*O*-rhamnoside) from its spectral data and comparison to previous work (Gaffield, Waiss Jr. and Tominaga, 1975; Silva, Yoshida and Kato, 1995). This flavanonol has been found in

several plants, such as *Eucryphia cordifolia* (Tschesche *et al.*, 1979), *Artocarpus altilis* (Chen *et al.*, 1993), *A. dadah* (Su *et al.*, 2002) and *Flindersia australis* (Reisch, Hussain and Mester, 1984). It was also found as a constituent in grape and wine (Trousadale and Singleton, 1983).

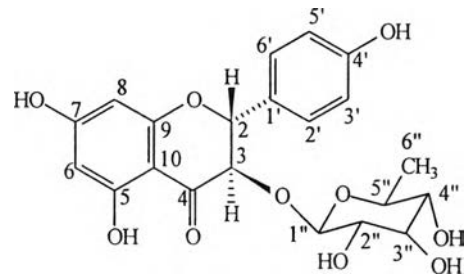

Table 28. Comparison of the ^{1}H and ^{13}C NMR spectral data of engeletin and compound SC-5 (in CD_3OD, 500 MHz)

Position	Engeletin		Compound SC-5		
	^{1}H†	^{13}C‡	^{1}H	^{13}C	HMBC
2	5.15 (1H, *d*, *J* = 11.5 Hz)	81.8	5.14 (1H, *d*, *J* = 10.8 Hz)	83.9	C-4, C-2′, C-3′, C-6′
3	4.25 (1H, *d*, *J* = 11.5 Hz)	76.3	4.62 (1H, *d*, *J* = 10.8 Hz)	78.7	C-2, C-4, C-1′, C-1″
4	-	195.4	-	196.1	-
5	-	163.7	-	164.2	-
6	5.90 (1H, *d*, *J* = 2.4 Hz)	96.4	5.89 (1H, *d*, *J* = 2.3 Hz)	96.3	C-5, C-7, C-8, C-10
7	-	167.4	-	168.6	-
8	5.97 (1H, *d*, *J* = 2.4 Hz)	95.4	5.92 (1H, *d*, *J* = 2.3 Hz)	97.4	C-6, C-9
9	-	162.5	-	165.5	-
10	-	101.3	-	102.5	-
5-OH	12.76 (1H, *s*)	-	-	-	-
7-OH	11.24 (1H, *s*)	-	-	-	-
1′	-	126.8	-	128.6	-
2′	7.35 (1H, *d*, *J* = 9.0 Hz)	129.4	7.35 (1H, *d*, *J* = 8.8 Hz)	130.1	C-4′, C-6′
3′	6.92 (1H, *d*, *J* = 9.0 Hz)	115.5	6.84 (1H, *d*, *J* = 8.8 Hz)	116.4	C-4, C-1′, C-5′
4′	8.23 (1H, *s*)	158.2	-	159.5	-
5′	6.92 (1H, *d*, *J* = 9.0 Hz)	115.5	6.84 (1H, *d*, *J* = 8.8 Hz)	116.4	C-4′, C-1′
6′	7.35 (1H, *d*, *J* = 9.0 Hz)	129.4	7.35 (1H, *d*, *J* = 8.8 Hz)	130.1	C-2, C-2′, C-4′
1″	-	100.6	4.01 (1H, *d*, *J* = 1.5 Hz)	102.2	C-3, C-3″, C-5″
2″	-	69.3	3.50 (1H, *dd*, *J* = 3.3, 1.5 Hz)	71.8	C-3″, C-4″
3″	-	70.4	3.65 (1H, *dd*, *J* = 10.0, 3.3 Hz)	72.2	C-2″, C-4″
4″	-	70.7	3.31 (1H, *dd*, *J* = 5.0, 1.5 Hz)	73.8	C-3″, C-5″
5″	-	71.9	4.25 (1H, *m*)	70.5	C-1″
6″	-	18.0	1.18 (3H, *d*, *J* = 6.5 Hz)	17.9	C-4″, C-5″

† Reisch *et al.*, 1984 (in DMSO-d_6, 90 MHz) ‡ Silva *et al.*, 1997 (in DMSO-d_6, 50 MHz)

2.6 Identification of Compound SC-6

Compound SC-6 was obtained as light brown crystals, soluble in MeOH. The UV spectrum (**Figure 121**) showed absorption maxima at 235, 291, and 335, and the IR spectrum (**Figure 122**) displayed prominent bands at 3419 (hydroxy group), 2935 (C-H stretching) and 1643 cm^{-1} (carbonyl group). Both the UV and IR spectra of compound SC-6 are similar to those of compound SC-5, suggesting they belong to the same group of compounds. The EI mass spectrum (**Figure 123**) showed molecular ion peak at *m/z* 450, corresponding to a molecular formula of $C_{21}H_{22}O_{11}$, which is 16 mass units more than that of compound SC-5, indicating an additional hydroxy function. The main mass fragment peak at *m/z* 286 corresponded to the loss of a hexose sugar unit.

The ^1H NMR spectrum of compound SC-6 (**Figure 124**) is closely similar to that of compound SC-5. The resonances at δ 5.07 and 4.57 (each 1H, *d*, J = 11.0 Hz) could be readily assigned to H-2 and H-3, respectively. The coupling constant of 11.0 Hz indicated a *trans*-configuration between H-3 and ring B of the flavonoid, which was further confirmed by NOESY experiment (**Figure 130**). Furthermore, the signals of H-6 at δ 5.89 and H-8 at δ 5.92 are *meta*-coupled to each other with a coupling constant of 2.5 Hz. The differences in the proton spectrum between these two compounds are the number of aromatic protons and their coupling pattern, especially those on ring B, suggesting different substitution pattern for this ring. For compound SC-6, two hydroxy groups could be located at C-3′ and C-4′, based on the spectral features of H-6′ (δ 6.84, *dd*, J = 8.0, 1.8 Hz), which was *meta*-coupled to H-2′ (δ 6.95, *d*, J = 1.8 Hz), while also *ortho*-coupled to H-5′ (δ 6.81, *d*, J = 8.0 Hz). These coupling could also be seen in the ^1H-^1H COSY spectrum (**Figure 127**).

The ^{13}C NMR spectral data (**Figure 125**) of compound SC-6 were also similar to those of compound SC-5, except for an additional quaternary carbon attached to a heteroatom (C-3′, δ 146.6) in place of a methine carbon in compound SC-5. All carbon signals were assigned according to the HMQC (**Figure 128**) and HMBC (**Figure 129**) spectra.

Based on these spectral data and comparison with earlier reports (Gaffield and Waiss, Jr., 1975; De Britto *et al.*, 1995), compound SC-6 was identifed as a dihydroflavonol rhamnoside called astilbin.

Astilbin, previously isolated from the leaves of *Engelhardtia chrysolepis,* a plant of which its leaves are used as sweet tea (Kasai *et al.*, 1988), was reported as an inhibitor of both rat lens and human recombinant aldose reductase enzyme (Haraguchi *et al.*, 1997), with an IC_{50} value of 26.7 μM. Kinetic analysis showed that astilbin uncompetitively inhibited lens aldose reductase with respect to both *dl*-glyceraldehyde and NADPH (Haraguchi *et al.*, 1996b). In addition, it has been demonstrated to protect against oxidative damage (Haraguchi *et al.*, 1996a). It was often isolated together with engeletin (compound SC-5) from the same source, e.g. *Eucryphia cordifolia* (Tschesche *et al.*, 1979) and grape pomace (Lu and Foo, 1999). Both of them can be found in beverages such as wine (Trousdale and Singleton, 1983) and champagne (Lu and Foo, 1999; Chamkha *et al.*, 2003).

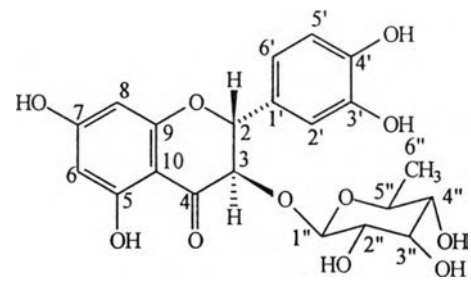

Table 29. Comparison of the ^{1}H and ^{13}C NMR spectral data of astilbin and compound SC-6 (in CD_3OD, 500 MHz)

Position	Astilbin†		Compound SC-6		
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC
2	5.21 (1H, *d*, *J* = 10.1 Hz)	84.8	5.07 (1H, *d*, *J* = 11.0 Hz)	84.0	C-3, C-4, C-1′, C-2′, C-6′
3	4.62 (1H, *d*, *J* = 10.1 Hz)	79.4	4.57 (1H, *d*, *J* = 11.0 Hz)	78.5	C-2, C-10, C-1′, C-1″,
4	-	196.7	-	196.0	-
5	-	166.3	-	164.1	-
6	5.86 (1H, *d*, *J* = 2.0 Hz)	98.2	5.89 (1H, *d*, *J* = 2.5 Hz)	96.3	C-5, C-7, C-8, C-10
7	-	169.7	-	168.6	
8	5.89 (1H, *d*, *J* = 2.0 Hz)	97.2	5.92 (1H, *d*, *J* = 2.5 Hz)	97.4	C-6, C-9, C-10
9	-	164.9	-	165.5	
10	-	103.2	-	102.5	
1′	-	130.0	-	129.2	
2′	6.89 (1H, *br s*)	117.1	6.95 (1H, *d*, *J* = 1.8 Hz)	115.5	C-2, C-4′, C-6′
3′	-	147.3	-	146.6	
4′	-	148.1	-	147.4	
5′	6.70-6.75 (1H, *m*)	116.3	6.81 (1H, *d*, *J* = 8.0 Hz)	116.2	C-1′, C-3′
6′	6.70-6.75 (1H, *m*)	121.3	6.84 (1H, *dd*, *J* = 8.0, 1.8 Hz)	120.5	C-2, C-2′, C-4′
1″	4.03 (1H, *d*, *J* = 1.0 Hz)	102.9	4.05 (1H, *d*, *J* = 1.5 Hz)	102.2	C-3, C-3″, C-5″
2″	3.35 (1H, *dd*, *J* = 3.7, 1.0 Hz)	72.6	3.54 (1H, *dd*, *J* = 3.5, 1.5 Hz)	71.8	C-3″, C-4″
3″	3.21 (1H, *dd*, *J* = 9.5, 3.7 Hz)	72.9	3.66 (1H, *dd*, *J* = 9.5, 3.5 Hz)	72.2	C-4″
4″	3.13 (1H, *t*, *J* = 9.5 Hz)	74.6	3.31 (1H, *dd*, *J* = 5.0, 2.0 Hz)	73.8	C-3″, C-5″
5″	3.91 (1H, *dq*, *J* = 9.5, 6.1 Hz)	71.3	4.25 (1H, *m*)	70.5	C-1″
6″	1.06 (3H, *d*, *J* = 6.1 Hz)	18.6	1.18 (3H, *d*, *J* = 6.5 Hz)	17.9	C-4″, C-5″

† de Britto *et al.*, 1995 (DMSO-d_6, 500 MHz)

2.7 Identification of Compound SC-7

Compound SC-7 was obtained as white amorphous powder. The UV absorption maxima (**Figure 131**) at 233, 243, 259 and 281 nm and the IR absorption bands (**Figure 132**) at 1517 and 1462 cm^{-1} were indicative of aromatic rings.

Analysis of signal integration for the ^1H NMR spectrum of compound SC-7 (**Figure 133a** and **133b**) indicated its true nature as a 2:1 mixture of two 2,5 diaryl-3,4-dimethyltetrahydrofuran lignans. Two set of proton signals could be determined from the correlations in its ^1H-^1H COSY spectrum (**Figure 135**). One set of these proton signals belongs to galgravin (or compound SC-1), which is a molecule with symmetrical chemical structure. This component of the mixture SC-7 exhibited signals at 0.97 (6H, *d*, J = 6.7 Hz, H-9, H-9′), whereas methine protons which appeared as a doublet at δ 4.47 (2H, J = 6.4 Hz) and a multiplet at δ 2.28 were assigned as H-7/H-7′ and H-8/H-8′, respectively. Its aromatic protons appeared as coupled resonances at δ 6.96 (2H, *d*, J = 1.8 Hz, H-2/H-2′), 6.93 (2H, *dd*, J = 7.5, 1.8 Hz, H-6/H-6′) and 6.79 (2H, *d*, J = 7.5 Hz, H-5/H-5′). The observed HMBC correlation (**Figure 137n**) between the signals of H-8 (or H-8′) at δ 2.28 and C-8′ (or C-8) at δ 44.1 confirmed the symmetrical structure of this minor component.

Another set of proton signals from the major component of this mixture could be divided into 3 groups: the first are those of the two connected C_3 units, and the other two are those of two aromatic rings with the same substitution pattern as those of galgravin. The doublets at δ 1.02 (3H, J = 6.4 Hz) and 0.61 (3H, J = 7.0 Hz) could be ascribed to C-9 and C-9′ methyl groups, respectively. The doublets at δ 4.37 (1H, J = 9.4 Hz) and 5.08 (1H, J = 8.5 Hz), assigned to H-7 and H-7′, were coupled to the multiplets at δ 1.74 (H-8) and 2.20 (H-8′), respectively. The aromatic protons of this component appeared as two set of three coupled resonances at δ 7.04 (1H, *d*, J = 2.0 Hz, H-2), 7.00 (1H, *dd*, J = 7.3, 2.0 Hz, H-6) and 6.82 (1H, *d*, J = 7.3 Hz, H-5), and at δ 6.85 (1H, *d*, J = 1.4 Hz, H-2′), 6.84 (1H, *dd*, J = 8.1, 1.4 Hz, H-6′) and 5.81 (1H, *d*, J = 8.1 Hz, H-5′). All protons and carbons were assigned according to the correlations in HMQC spectrum (**Figure 136a-136c**). The NOESY experiment (**Figure 138a** and **138b**) showed the correlations between H-8′ to both H-7′ and H-9, H-7 to both H-7′ and H-9 and between H-8 and H-9′. This set of proton signals belongs to veraguensin, which is a stereoisomer of galgravin.

Based on the above spectral evidence and comparison with previously reported data, compound SC-7 was identified as a mixture of two lignan stereoisomers, veraguensin and galgravin, in the ratio of 2:1.

Veraguensin can be found in the genus *Magnolia* of the family Magnoliaceae such as *Magnolia acuminata* (Doskotch and Flom, 1972), *M. liliflora* (Iida and Ito, 1983) and *M. denudata* (Du *et al.*, 2001). It was also isolated from members of the family Lauraceae e.g. *Ocotea veraguensis* (Khan *et al.*, 1987) and *Nectandra puberula* (Moro *et al.*, 1987), and from the fruits and leaves of *Illicium floridanum* (family Illiciaceae) (Schmidt and Heilmann, 2000). Veraguensin, isolated from the twigs of *Virola surinamensis* (family Myristicaceae), was very potent against *Trypanocidal cruzi* in the trypomatigote form (Nihei *et al.*, 2000).

The co-occurrence of veraguensin and galgravin in the same plant has previously been reported from the studies of *Litsea grandis* and *L. gracilipes* (Holloway and Scheinmann, 1974), *Aristolochia arcuta* (Zhai *et al.*, 2005) and *Nectandra megapotamica* (Filho *et al.*, 2004).

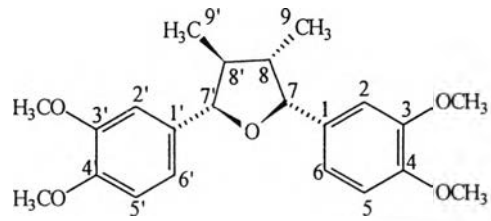

Table 30. Comparison of the ^{1}H and ^{13}C NMR spectral data of veraguensin and the major component of mixture SC-7 (in $CDCl_3$, 500 MHz)

Position	Veraguensin		Major component of SC-7		
	^{1}H†	^{13}C‡	^{1}H	^{13}C	HMBC
1	-	133.2	-	133.3	
2	6.80-7.20 (1H, *m*)	109.7	7.04 (1H, *d*, *J* = 2.0 Hz)	109.7	C-4, C-6, C-7
3	-	148.3	-	148.7	
4	-	147.8	-	148.4	
5	6.80-7.20 (1H, *m*)	110.5	6.82 (1H, *d*, *J* = 7.3 Hz)	110.9	C-1
6	6.80-7.20 (1H, *m*)	118.4	7.00 (1H, *dd*, *J* = 7.3, 2.0 Hz)	118.5	C-2, C-4
7	4.43 (1H, *d*, *J* = 8.6 Hz)	82.8	4.37 (1H, *d*, *J* = 9.5 Hz)	87.0	C-6, C-9
8	1.70 (1H, *m*)	47.8	1.74 (1H, *m*)	47.7	C-1, C-7, C-8′
9	1.06 (3H, *d*, *J* = 6.2 Hz)	14.9	1.02 (3H, *d*, *J* = 6.4 Hz)	14.8	C-7, C-8, C-8′
1′	-	133.6	-	133.6	
2′	6.80-7.20 (1H, *m*)	110.5	6.85 (1H, *d*, *J* = 1.4 Hz)	110.2	C-6′, C-7′
3′	-	148.7	-	148.7	
4′	-	148.3	-	148.4	
5′	6.80-7.20 (1H, *m*)	110.8	5.81 (1H, *d*, *J* = 8.1 Hz)	110.8	
6′	6.80-7.20 (1H, *m*)	119.1	6.84 (1H, *dd*, *J* = 8.1, 1.4 Hz)	119.0	C-1′, C-2′
7′	5.16 (1H, *d*, *J* = 8.2 Hz)	87.1	5.08 (1H, *d*, *J* = 8.5 Hz)	82.8	C-2′, C-6′, C-9′
8′	2.20 (1H, *m*)	45.9	2.20 (1H, *m*)	45.8	C-1′, C-7′, C-8, C-9′
9′	0.66 (3H, *d*, *J* = 6.7 Hz)	14.9	0.61 (3H, *d*, *J* = 7.0 Hz)	14.8	C-7′, C-8′, C-8
3-OCH_3	3.88, 3.92	55.7	3.81 (3H, *s*)*, 3.82 (3H, *s*)*, 3.83 (3H, *s*)*, 3.86 (3H, *s*)*	55.6*, 55.7*	
4-OCH_3					
3′-OCH_3					
4′-OCH_3					

† Doskotch and Flom, 1972 *may be interchangeable

‡ Fonseca *et al.*, 1979

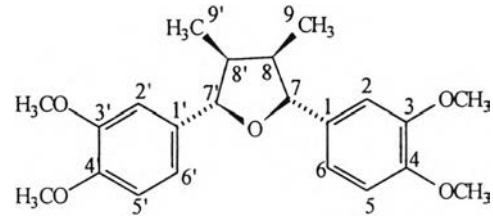

Table 31. Comparison of the ^{1}H and ^{13}C NMR spectral data of galgravin and the minor component of mixture SC-7 (in $CDCl_3$, 500 MHz)

Position	Galgravin		Minor Component of SC-7		
	^{1}H†	^{13}C‡	^{1}H	^{13}C	HMBC
1	-	134.8	-	133.7	
2	7.00 (1H, *m*)	109.7	6.96 (1H, *d*, *J* = 1.8 Hz)	109.5	C-1, C-4, C-6, C-7
3	-	148.9	-	147.8	
4	-	148.4	-	148.4	
5	7.00 (1H, *m*)	110.9	6.79 (1H, *d*, *J* = 7.5 Hz)	110.5	C-3
6	7.00 (1H, *m*)	118.5	6.93 (1H, *dd*, *J* = 7.5, 1.8 Hz)	118.4	C-2, C-4
7	4.53 (1H, *m*)	87.2	4.47 (1H, *d*, *J* = 6.4 Hz)	87.0	C-2, C-6, C-9
8	2.40 (1H, *m*)	44.3	2.28 (1H, *m*)	44.1	C-1, C-7, C-8′
9	1.05 (3H, *d*)	12.9	0.97 (3H, *d*, *J* = 6.7 Hz)	12.7	C-7
1′	-	134.8	-	133.7	
2′	7.00 (1H, *m*)	109.7	6.96 (1H, *d*, *J* = 1.8 Hz)	109.5	C-1′, C-4′, C-6′, C-7′
3′	-	148.9	-	147.8	
4′	-	148.4	-	148.4	
5′	7.00 (1H, *m*)	110.9	6.79 (1H, *d*, *J* = 7.5 Hz)	110.5	
6′	7.00 (1H, *m*)	118.5	6.93 (1H, *dd*, *J* = 7.5, 1.8 Hz)	118.4	C-2′, C-4′
7′	4.53 (1H, *m*)	87.2	4.47 (1H, *d*, *J* = 6.4 Hz)	87.0	C-2′, C-6′, C-9′
8′	2.40 (1H, *m*)	44.3	2.28 (1H, *m*)	44.1	C-1′, C-7′, C-8
9′	1.05 (3H, *d*)	12.9	0.97 (3H, *d*, *J* = 6.7 Hz)	12.7	C-7′, C-8
3- OCH_3	3.87 (3H, *s*)	55.8*, 55.9*	3.81 (3H, *s*)*, 3.82 (3H, *s*)*, 3.83 (3H, *s*)*, 3.86 (3H, *s*)*	55.6*, 55.7*	
3′-OCH_3	3.87 (3H, *s*)				
4- OCH_3	3.87 (3H, *s*)				
4′-OCH_3	3.87 (3H, *s*)				

† Holloway and Scheinmann, 1974 (in $CDCl_3$, 60 MHz)

‡ Kuo *et al.*, 2002 (in $CDCl_3$, 75 MHz) *interchangeable in the same column

2.8 Identification of compound SC-8

Compound SC-8 was obtained as pale yellow amorphous powder, soluble in MeOH. The EIMS spectrum (**Figure 141**) of compound SC-8 exhibited a molecular ion peak at *m/z* 265, consistent with a molecular formula of $C_{16}H_{11}NO_3$, and also showed other major peaks at *m/z* 250 $[M\text{-}Me]^+$, 222 $[M\text{-}CO\text{-}Me]^+$. The UV spectrum (**Figure 139**) demonstrated absorption maxima at 276, 342, 356, 365 and 383 nm characteristic of phenanthrene chromophore. The IR spectrum (**Figure 140**) showed absorption bands at 3600-3188 (OH and NH streching) and 1698 (C=O streching) cm^{-1}, suggesting a lactam group.

The ^1H NMR spectrum (**Figure 142**) of this compound exhibited one singlet signal integrating for three protons at δ 4.04, six aromatic protons at δ 9.25, 7.93, 7.76, 7.53 (2 protons), 7.12 and the most downfield signal at δ 10.65 (1H, *s*) which was assigned to the secondary amide proton. The ^{13}C NMR spectrum (**Figure 143**) showed 16 carbon signals, classified by DEPT experiment into those of one carbonyl carbon (the most downfield signal), one methoxy group, six methine carbons and nine quaternary carbons. Two of the quaternary carbon signals were deshielded by the inductive effect of the attached oxygen atoms.

Two aromatic proton singlets at δ 7.76 and 7.12 could be assigned to H-2 on ring A and H-9 on ring C, respectively. Conjugated signals at δ 9.25 (1H, *d*, J = 7.5 Hz), 7.93 (1H, *d*, J = 7.8 Hz) and 7.53 (2H, *m*) suggested four adjacent aromatic protons. The most downfield aromatic resonance at δ 9.25 is characteristic of H-5, the two-proton signal at δ 7.53 were assigned to H-6 and H-7, and the doublet at δ 7.93 was due to H-8 of ring D of the aristolactam structure. Therefore, one methoxy group should be substituted on ring A. The methoxy proton signal at δ 4.04 showed long- range correlation to the carbon signal at δ 150.3, which was also correlated with H-2 signal in the HMBC spectrum (**Figure 146a-146c**), indicating the position of that methoxy group as at C-3.

On the basis of the above evidences, compound SC-8 was identified as piperolactam A (10-amino-4-hydroxyl-3-methoxyphenanthrene-1-carboxylic acid lactam). The assignments of NMR resonances were confirmed by HSQC (**Figure 145a** and **145b**) and HMBC techniques.

Piperolactam A was found not only in *Fissistigma glaucescens* (Lo, Chang and Wu, 2000) and *Fissistigma balansae* (Chia *et al.*, 2000) of the family

Annonaceae, but also in the roots of *Piper longum,* which is the first report of the isolation of aristolactams from the family Piperaceae (Desai, Prabhu and Mulchandani, 1988). Additionally, it could be found in several other *Piper* species e.g. *P. argyrophyllum* (Singh *et al.*, 1996), *P. hamiltonii*, *P. wightii*, *P. attenuatum*, and *P. boehimerifolium* (Kumar *et al.*, 2003). It was also isolated from *Houttuynia cordata* of the family Saururaceae (Probstle and Bauer, 1992). Investigation of piperolactam A isolated from *Piper sanctum* showed that the compound could inhibit the growth of *Mycobacterium tuberculosis* with MIC value of 8 μg/ml (Mata *et al.*, 2004). The alkaloid, isolated from *Fissistigma bracteolatum*, displayed potential anti-inflammatory activity by inhibiting nitric oxide (NO) generation by RAW-264.7 macrophage (Lan *et al.*, 2005).

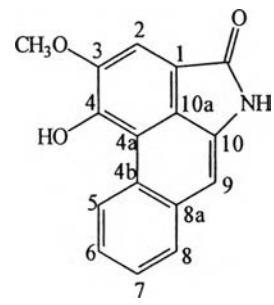

Table 32. Comparison of NMR spectral data of piperolactam A and compound SC-8 (in DMSO-d_6, 300 MHz)

Position	Piperolactam A	Compound SC-8		
	^1H†	^1H	^{13}C	HMBC
1	-	-	116.8	
2	7.76 (1H, *s*)	7.76 (1H, *s*)	109.4	C-1, C-3*, C-4, C-10a, C=O
3	-	-	149.1	
4	-	-	150.3	
4a	-	-	115.3	
4b	-	-	127.5	
5	9.32 (1H, *m*)	9.25 (1H, *d*, *J* = 7.5)	128.3	C-4b, 7, 8a
6	7.54 (2H, *m*)	7.53 (2H, *m*)	125.2	C-4b, C-5*, C-7*, C-8
7	7.54 (2H, *m*)	7.53 (2H, *m*)	127.5	C-5, C-8a
8	7.86(1H, *m*)	7.93 (1H, *d*, *J* = 7.8)	129.6	C-4b, C-6, C-7, C-9
8a	-	-	135.0	
9	7.15 (1H, *m*)	7.12 (1H, *s*)	105.3	C-4b, C-8,C-8a*, C-10, C-10a
10	-	-	135.4	
10a	-	-	125.1	
3-OCH_3	4.09 (3H, *s*)	4.04 (3H, *s*)	58.1	
4-OH	-	10.70 (1H, *s*)	-	C-3, C-4*
C=O	-	-	169.8	
NH	-	10.65 (1H, *s*)	-	C-1, C-10*, C-10a, C=O

† Lo, Chang and Wu, 2000 (in $CDCl_3$, 200 MHz,)

* Two bond coupling

2.9 Identification of Compound SC-9

Compound SC-9 was obtained as yellow amorphous powder, soluble in MeOH. Its EIMS (**Figure 149**) displayed molecular ion peak at *m/z* 265, corresponding to $C_{16}H_{11}NO_3$. Mass fragment peaks at m/z 250 and 222 correspond to losses of a methyl group, followed by a carbonyl moiety. Its UV spectrum (**Figure 147**) showed characteristic maxima of a phenanthrene chromophore at λ_{max} 248, 272, 281, 370 and 388 nm. The IR spectrum (**Figure 148**) revealed maximal absorptions at 3600-3200 (OH and NH stretching), 1702 (C=O stretching) and 1293 (C-O stretching) cm^{-1}. These features suggested the presence of lactam and hydroxyl moieties. This compound shared many structural features in common with compound SC-8.

Examination of the ^{13}C NMR (**Figure 151**) displayed the signals of one methoxy group at δ 59.6, six methine carbons at δ 104.0, 113.5, 125.4, 126.9, 127.4 and 129.1, eight quaternary carbons at δ 120.5, 121.9, 122.5, 126.1, 134.9, 135.4, 149.0 and 152.3, and one carbonyl carbon at δ 168.6. The ^1H NMR spectrum of compound SC-9 (**Figure 150**), which revealed the presence of one methoxy signal at δ 4.00 (3H, *s*), six aromatic proton signals at δ 7.09 (1H, *s*), 7.54 (2H, *m*), 7.61 (1H, *s*), 7.92 (1H, *d*, J = 7.8 Hz) and 9.10 (1H, *d*, J = 7.5 Hz), and a secondary amide signal at δ 10.78 (1H, *s*), is very similar to that of compound SC-8. The difference is in the aromatic proton signal of H-2 at δ 7.61, which appeared more upfield compared to similar proton in compound SC-8. This could be due to shielding effect from hydroxy substitution at C-3. Hence, the methoxy group could reasonably be placed at C-4. This was confirmed by NOESY experiment (**Figure 155**), in which the signal of H-5 at δ 9.10 gave a cross-peak with the methoxy signal at δ 4.00.

Based on the information obtained from the HMQC (**Figure 153**) and HMBC spectra (**Figure 154a-154c**), all protons and carbons in the chemical structure of compound SC-9 were assigned.

From the above spectral analysis and comparison with previously reported data (Likhitwitayawuid *et al.*, 1997), compound SC-9 was identified as aristolactam AII. This alkaloid was first isolated from the roots of *Aristolochia argentina* (Crohare *et al.*, 1974), and later from *A. indica* (family Aristolochiaceae) (Achari *et al.*, 1982). In plants of the family Annonaceae, it was previously found in *Goniothalamus sesquipedalis* (Talapatra *et al.*, 1988), *Annona cacans* (Saito and Alvarenga, 1994), *G.*

tenuifolius (Likhitwitayawuid *et al.*, 1997), *G. griffithii* (Zhang *et al.*, 1999), *Fissistigma balansae, F. oldhamii* (Chia *et al.*, 2000) and *F. bracteolatum* (Lan *et al.*, 2005). The compound was shown to exhibit antimalarial activity with IC_{50} value of 9.54 μg/ml (Likhitwitayawuid *et al.*, 1997).

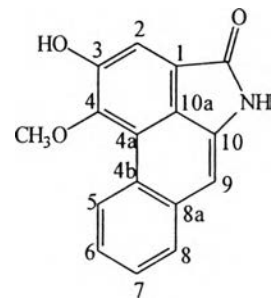

Table 33. Comparison of the NMR spectral data of aristolactam AII and compound SC-9 (in DMSO-d_6, 300 MHz)

Position	Aristolactam AII†		Compound SC-9		
	^1H	^{13}C	^1H	^{13}C	HMBC
1	-	121.8	-	121.9	
2	7.61 (1H, *s*)	113.4	7.61 (1H, *s*)	113.5	C-3, C-4, C-10a, C=O
3	-	152.3	-	152.3	
4	-	149.8	-	149.0	
4a	-	120.4	-	120.5	
4b	-	125.4	-	126.1	
5	9.09 (1H, *dd, J* = 7.8 Hz, 1.5)	126.8	9.10 (1H, *d, J* = 7.5 Hz)	126.9	C-8a, C-7, C-4a
6	7.54 (1H, *ddd, J* = 7.8, 7.8, 1.5 Hz)	125.3	7.54 (1H, *m*)	125.4	C-4b, C-8
7	7.56 (1H, *ddd, J* = 7.8, 7.8, 1.5 Hz)	127.3	7.54 (1H, *m*)	127.4	C-8a, C-5
8	7.93 (1H, *dd, J* = 7.8, 1.5 Hz)	129.0	7.92 (1H, *d, J* = 7.8 Hz)	129.1	C-6, C-4b
8a	-	134.9	-	134.9	
9	7.08 (1H, *s*)	103.9	7.09 (1H, *s*)	104.0	C-8, C-8a*, C-10a, C-4b
10	-	135.3	-	135.4	
10a	-	122.3	-	122.5	
3-OH	-	-	-	-	
4-OCH_3	4.01 (3H, *s*)	59.5	4.00 (3H, *s*)	59.6	C-4
C=O	-	168.5	-	168.6	
NH	10.78 (1H, *s*)	-	10.78 (1H, *s*)	-	C-1, C-10, C-10a, C=O

† Likhitwitayawuid *et al.*, 1997 (in DMSO-d_6, 500 MHz)

2.10 Identification of compound SC-10

Compound SC-10 was obtained as pale yellow amorphous powder, giving molecular ion peak at *m/z* 295 in the ESITOF mass spectrum (**Figure 158**). Its tentative molecular formula was thus suggested as $C_{17}H_{13}NO_4$. The IR spectrum (**Figure 157**) indicated the presence of OH and NH (3248 cm^{-1}) and conjugated carbonyl functional groups (1610 cm^{-1}). Its UV spectrum (**Figure 156**) exhibited maximum absorption at λ_{max} 247, 273, 281 and 389 nm, characteristic of a phenanthrene chromophore.

Compound SC-10 is another aristolactam alkaloid, of which its ^1H NMR spectrum (**Figure 159**) displays a one-proton singlet at δ 7.12 ascribable to H-9, while a group of complex coupling signals further downfield represents H-5 - H-8. H-5 appeared as the most downfield doublet of doublets at δ 9.15 (J = 6.0, 3.3 Hz). Another doublet of doublets integrated for 2 protons at δ 7.50 (J = 6.0, 3.3 Hz) could be assigned to H-6 and H-7, while the middle one at δ 7.82 (1H, J = 6.0, 3.3 Hz) was due to H-8. ^1H-^1H COSY experiment (**Figure 162**) confirmed these assignments.

The ^1H NMR spectrum also displayed two methoxy singlets at δ 4.09 and 4.43. Since the previously mentioned aromatic protons represents a 5,6,7,8,9-unsubstituted aristolactam, both methoxy groups should therefore be located on ring A. The proton signals of methoxy substituted at C-3 and C-4 of phenanthrene lactams have been reported to resonate between δ 4.0-4.1 (Talapatra *et al.*, 1988). In the case of compound SC-10, one methoxy group gave a signal at a significantly downfield chemical shift of δ 4.43 from the deshielding effect of the *peri* carbonyl of the lactam group (Talapatra *et al.*, 1988), indicating its location as at C-2. NOESY experiment (**Figure 165**) was carried out to determine the position of the other methoxy group. The absence of any correlation between H-5 signal and both methoxy groups suggested that the second methoxy group should be located at C-3. All proton and carbon assignments were accomplished based on the HMQC (**Figure 163**) and HMBC (**Figure 164**) spectra .

Based on the spectral analysis and comparison to previous data (Olsen *et al.*, 1993), compound SC-10 was shown to be piperolactam D (10-amino-4-hydroxy-2,3-dimethoxyphenanthrene-1-carboxylic acid lactam). The structure of this compound isolated from *Piper longum* (Desai *et al.*, 1988), *P. attenuatum, P. boehmerifolium*

(Desai, Chaturvedi and Mulchandani, 1990) and *P. hamiltonii* (Desai *et al.*, 1989) was initially misinterpreted as that of piperolactam B (10-amino-2-hydroxy-3,4-dimethoxyphenanthrene-1-carboxylic acid lactam) from the confusion of methoxy substitution at C-2, C-3 or C-4. The assignment was revised after NOESY experiment was carried out (Olsen *et al.*, 1993).

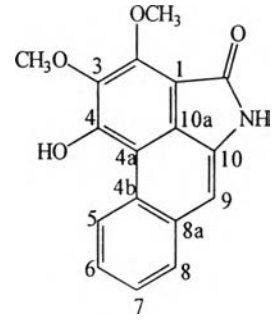

Table 34. Comparison of the NMR spectral data of piperolactam D and compound SC-10 (in CD_3OD, 300 MHz)

Position	Piperolactam D†		Compound SC-10		
	^1H	^{13}C	^1H	^{13}C	HMBC
1	-	111.6	-	117.6	
2	-	153.1	-	150.0	
3	-	154.6	-	151.4	
4	-	139.6	-	145.2	
4a	-	105.6	-	110.3	
4b	-	126.9	-	127.8	
5	9.25 (1H, *m*)	126.4*	9.15 (1H, *dd*, *J* = 6.0, 3.3 Hz)	127.5	C-4a, C-5, C-7, C-8a
6	7.53 (1H, *m*)	126.1	7.50 (1H, *dd*, *J* = 6.0, 3.3 Hz)	126.3	C-8
7	7.53 (1H, *m*)	126.6*	7.50 (1H, *dd*, *J* = 6.0, 3.3 Hz)	127.5	C-6, C-8a
8	7.95-7.53 (1H, *m*)	128.9	7.82 (1H, *dd*, *J* = 6.0, 3.3 Hz)	129.8	C-6, C-8a
8a	-	133.1	-	135.3	
9	7.23 (1H, *s*)	105.4	7.12 (1H, *s*)	106.1	C-6, C-8, C-8a, C-10a
10	-	134.6	-	135.7	
10a	-	125.4	-	123.4	
2-OCH_3	4.40 (3H, *s*)	62.4	4.43 (3H, *s*)	63.3	C-2
3-OCH_3	3.86 (3H, *s*)	61.1	4.09 (3H, *s*)	60.4	C-3
4-OH	10.78 (1H, *s*)	-	-	-	
C=O	-	167.1	-	169.4	
NH	10.68 (1H, *s*)	-	-	-	

† Olsen *et al.*, 1993 (in DMSO-d_6) * may be interchangeable

2.11 Identification of compound SC-11

Compound SC-11 was obtained as orange amorphous powder. Its UV spectrum (**Figure 166**) exhibits typical phenanthrene absorption maxima at λ_{max} 244, 284, 315 and 444 nm. Its mass spectrum (**Figure 168**) showed an $[M]^+$ peak at *m/z* 293, thus giving a molecular formula of $C_{17}H_{11}NO_4$. Compared to the mass spectrum of compound SC-8, the 28 mass unit higher molecular ion of compound SC-11 suggested an additional carbonyl group in the latter. The IR spectrum (**Figure 167**) of compound SC-11, in addition to displaying the presence of hydroxy group at 3367 cm^{-1}, revealed two carbonyl absorption peaks at 1679 and 1658 cm^{-1}.

The presence of two carbonyl groups was further evidenced from its ^{13}C NMR spectrum (**Figure 170**) which displayed resonances at δ 177.1 and 155.8. When analyzed together with DEPT 135 experiment (**Figure 171**), the carbon signals of one methoxy group (at δ 59.7), six methine carbons (at δ 112.1, 117.2, 126.8, 126.8, 128.0 and 128.5) and eight quaternary carbons (at δ 117.3, 124.2, 124.9, 126.2, 130.5, 132.6, 151.3 and 153.0) could also be differentiated. The ^1H NMR spectrum (**Figure169**) exhibited the most downfield signal of NH proton at δ 12.01, one methoxy signal at δ 4.04 (3H, *s*), two aromatic singlets at δ 7.48 and 8.07, and four conjugated aromatic protons at δ 7.63 (2H, *m*), 7.91 (1H, *m*) and 9.44 (1H, *m*) ascribed to the unsubstituted ring D of the dioxoaporphine skeleton. The methoxy group should therefore be located on ring A. The similarity of the NMR spectra of this compound to those of compound SC-9 suggested that their substitution pattern might be the same. NOESY experiment (**Figure 175**) exhibited cross peak between H-11 at δ 9.44 and methoxy signal at δ 4.04, indicating methoxy substitution at C-1 of the molecule.

Compound SC-11 was therefore identified as noraristolodione by the analysis of NMR spectra, including HMQC (**Figure 173**) and HMBC (**Figure 174a** and **174b**) experiments.

Noraristolodione is a 4,5-dioxoaporphine alkaloid isolated for the first time from *Aristolochia indica* of the family Aristolochiaceae (Achari *et al.*, 1982). It was also reported as a constituent of plants in the families Piperaceae e.g. *Piper longum* (Desai, Prabhu and Mulchandani, 1988) and Annonaceae e.g. *Fissistigma glaucescens* (Lo, Chang and Wu, 2000) and *F. balansae* (Chia *et al.*, 2000).

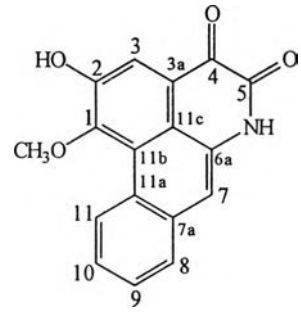

Table 35. Comparison of the ^{1}H and ^{13}C NMR spectral data of noraristolodione and compound SC-11 (in DMSO-d_6, 300 MHz)

Position	Noraristolodione		Compound SC-11		
	^{1}H†	^{13}C‡	^{1}H	^{13}C	HMBC
1	-	154.1	-	153.0	
2	-	152.8	-	151.3	
3	8.10	118.4	8.07 (1H, *s*)	117.2	C-1, C-4
3a	-	126.4	-	124.9	
4	-	178.3	-	177.1	
5	-	157.0	-	155.8	
6a	-	131.8	-	130.5	
7	7.50	112.3	7.48 (1H, *s*)	112.1	C-6a, C-8, C-11a
7a	-	133.6	-	132.6	
8	7.95 (1H, *d*)	128.9	7.91 (1H, *m*)	128.5	C-10
9	7.66 (1H, *m*)	128.2	7.63 (1H, *m*)	126.8	
10	7.66 (1H, *m*)	126.9	7.63 (1H, *m*)	128.0	
11	9.47 (1H, *d*)	128.1	9.44 (1H, *m*)	126.8	C-10, C-11b
11a	-	127.2	-	126.2	
11b	-	125.3	-	124.2	
11c	-	118.4	-	117.3	
1-OCH_3	4.06 (3H, *s*)	59.9	4.04 (3H, *s*)	59.7	C-1
2-OH	-	-	-	-	
NH	12.10	-	12.01	-	

† Desai, Prabhu and Mulchandani, 1988 (in DMSO-d_6, 500 MHz)

‡ Achenbach, Frey and Waibel, 1991 (in C_5D_5N, 90 MHz)

3. Bioactivity evaluation of compounds isolated from *Ellipeiopsis cherrevensis* and *Stelechocarpus cauliflorus*

In the search for biologically active constituents of *Ellipeiopsis cherrevensis* and *Stelechocarpus cauliflorus*, the hexane, $CHCl_3$ and MeOH extracts of the leaves and stems of *E. cherrevensis,* as well as the hexane, EtOAc and MeOH extracts of the leaves and stems of *S. cauliflorus,* were subjected to *in vitro* screenings for their anticancer activity against three cancer cell lines (KB, BC and NCI-H187), antimalarial activity against *Plasmodium falciparum*, antituberculosis activity against *Mycobacterium tuberculosis*, anti-herpes simplex virus type 1 and immunomodulating activities through their ability to stimulate or suppress lymphocyte proliferation. In addition, screening of all crude extracts for potential aldose reductase (AR) and advance glycation end-products (AGEs) formation inhibitors were also performed.

3.1 Bioactive compounds from *Ellipeiopsis cherrevensis*

The $CHCl_3$ extract of *E. cherrevensis* leaves was strongly active against NCI-H187 and BC cell lines with IC_{50} values of 0.02 and 2.35 μg/ml, respectively, but was weakly active against KB cell line with IC_{50} value of 15.03 μg/ml. It also displayed antituberculosis activity with MIC value of 50 μg/ml. The MeOH extract of the leaves was strongly active against NCI-H187 (IC_{50} = 2.1 μg/ml) and weakly active against KB cell lines (IC_{50} = 10.05 μg/ml). In addition, the $CHCl_3$ extract of the stems was strongly active against all three tested cell lines, with IC_{50} value against NCI-H187, KB and BC cell lines of 0.01, 0.03 and 0.08 μg/ml, respectively. This extract also exhibited antituberculosis (MIC value of 25 μg/ml) and antimalarial activities (EC_{50} = 10.9 μg/ml).

Subsequent extraction of the $CHCl_3$ extract of the leaves led to the isolation of three cyclohexene derivatives, which are ferrudiol, ellipieopsol D and zeylenol, and a rare flavonoid, 2′,4′-dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone. Two flavonoid glycosides, tiliroside and keampferol-3-*O*-rutinoside, were isolated from the MeOH extract of the leaves. Finally, an oxoaporphine alkaloid, lanuginosine, was isolated from the $CHCl_3$ extract of the stems. All compounds were evaluated for their biological activities, the results of which are summarized in **Table 36**.

3.1.1 Cytotoxicity activity

C-benzylated chalconoids have been reported as exhibiting cytotoxicity against several cancer cell lines (Cole, Torrance and Wiedhopf, 1976; Hufford and Lasswell, 1976). In the case of the $CHCl_3$ of this plant leaves, the C-benzylated chalcone, 2′,4′-dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone, represented the cytotoxic constituent of the extract. However, judging from the strong activity of the extract, there might be some other active constituents not isolated in this study. The chalcone exhibited strong cytotoxictiy against NCI-H187 cell line (IC_{50} = 1.40 μg/ml), moderate activity against KB cell line (IC_{50} = 5.31 μg/ml) and weak cytotoxic effect against BC cell lines (IC_{50} = 13.92 μg/ml). However, it was also cytotoxic against Vero cells (IC_{50} = 3.00 μg/ml), therefore its prospect for use as an anticancer agent might be limited.

Zeylenol, which was found to show antitumour activity against A549 bronchogenic carcinoma cell (Xu *et al.*, 2005), was inactive against the three cancer cell lines used in this study.

3.1.2 Antimalarial activity

2′,4′-Dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone displayed antimalarial activity with EC_{50} value of 7.1 μg/ml. Previously, C-benzylated dihydrochalcones uvaretin and diuvaretin, have been reported as possessing this activity with IC_{50} values of 3.49 and 4.20 μg/ml (Khunya *et al.*, 1991).

3.1.3 Antibuberculosis activity

Ellipieopsol D, zeylenol, lanuginosine and 2′,4′-dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone exhibited antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Ra$ with MICs values of 200, 100, 100 and 25 μg/ml, respectively.

3.1.4 Anti HSV-1 acitivity

Although none of the extracts from this plant displayed any detectable anti HSV-1 activity, when the isolated constituents were subjected to the assay, zeylenol showed moderate anti HSV-1 activity, whereas ferrudiol and 2′,4′-

dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone were weakly active. For ferrudiol, this is the only report of its bioactivity from this study.

3.1.5 Lymphocyte proliferation stimulating activity

The polyoxygenated cyclohexenes, ferrudiol and zeylenol, together with the flavonoid glycosides, tiliroside and kaempferol-3-*O*-rutinoside, were able to stimulate lymphocyte proliferation with stimulation index (SIs) of 1.41, 1.24, 1.46 and 1.40, respectively. On the contrary, the alkaloid lanuginosine suppressed lymphocyte proliferation.

Table 36. Bioactive activities of isolated compounds from *Ellipeiopsis cherrevensis*

Compound	SI*	Anti HSV-1 IC_{50} (μg/ml)	AntiTB MIC (μg/ml)	Antimalarial activity EC_{50} (μg/ml)	Cytotoxicity IC_{50} (μg/ml)			
					NCI-H187	KB	BC	Vero cell
Ferrudiol (EC-1)	1.41	weakly active	inactive	inactive	inactive	inactive	inactive	>50
2′,4′-dihydroxy-3′-(2-hydroxybenzyl)-6′-methoxychalcone (EC-2)	ND	weakly active	25	7.1	1.40	5.31	13.92	3.0
Ellipeiopsol D (EC-3)	ND	ND	200	inactive	inactive	inactive	inactive	>50
Zeylenol (EC-4)	1.24	moderately active	100	inactive	inactive	inactive	inactive	>50
Tiliroside (EC-5)	1.46	inactive	inactive	inactive	inactive	inactive	inactive	>50
Kaempferol-3-*O*-rutinoside (EC-6)	1.40	inactive	inactive	inactive	inactive	inactive	inactive	>50
Lanuginosine (EC-7)	0.57	inactive	100	inactive	inactive	inactive	inactive	>50
Concanavalin A	2.04	-	-	-	-	-	-	-
Rifampicin	-	-	0.0047	-	-	-	-	-
Kanamycin sulfate	-	-	2.5	-	-	-	-	-
Isoniazid	-	-	0.05	-	-	-	-	-
Ellipticine	-	-	-	-	0.35	0.38	0.32	-
Doxorubicin	-	-	-	-	-	0.17	0.18	-
Dihydroartemisin	-	-	-	0.0043	-	-	-	-
Acyclovir	-	0.9-1.9	-	-	-	-	-	-

ND = not determined *at 12.5 μg/ml ** at 2.5 μg/ml

3.2 Bioactive compounds from *Stelechocarpus cauliflorus*

The hexane extract of the leaves and the EtOAc extract of the stems of *S. cauliflorus* showed anti HSV-1 activity with IC_{50} values of 11.4 and 14.2 μg/ml, respectively. In addition, the hexane extract of leaves and both the hexane and EtOAc extracts of the stems were active against *Mycobacterium tuberculosis* with equal MIC value of 200 μg/ml. When subjected to initial screening for the ability to prevent diabetic complications, the EtOAc extract of the leaves was found to possess interesting inhibitory activities against AR and AGEs formation (58.4% and 64.6% inhibition at 5 μg/mL, respectively).

Chemical investigation of the hexane extract of the leaves led to the isolation of three lignans, which are galgravin, licarin A and acuminatin, together with a 2:1 mixture of veraguensin (another lignan) and galgravin. The EtOAc extract of the stems afforded four oxoaporphine alkaloids: piperolactams A and D, aristolactam A II and noraristolodione. Fractionation based on activities against AR and AGEs formation yielded engeletin and astilbin as the active components. The results of the bioactivity evaluation of these isolated compounds are summarized in **Table 37**.

3.1.1 Cytotoxicity activity

The neolignan licarin A displayed strong cytotoxic activity against BC cell line (IC_{50} = 4.06 μg/ml), moderate cytotoxicity against KB cell line (IC_{50} = 5.07 μg/ml) and weak activity against NCI-H187 cell line (IC_{50} = 13.67 μg/ml), whereas acuminatin, which is another neolignan, exhibited weak cytotoxicity against KB cell line (IC_{50} = 16.32 μg/ml). The activity against KB cell line of licarin A was in agreement with a previous study by Quesne *et al.* (1980), which reported its ED_{50} as 7.0 μg/ml. On the other hand, acuminatin was shown to be weakly cytotoxic against colon carcinoma (HT-29) and human breast carcinoma (MCF-7) cell lines (Li *et al.*, 2004).

The structures of licarin A and acuminatin are rather similar except for the different substitution at C-4′, in which the hydroxy group of licarin A was replaced by a methoxy group in acuminatin, suggesting the importance of hydroxy substitution for this activity.

The aporphine alkaloid piperolactam D was also active against two cancer cell lines. It was found to express moderate cytotoxic activity to KB and BC cell lines, with Ics_{50} of 6.43 and 5.52 μg/ml, respectively.

Table 37. Bioactivities of isolated compounds from *Stelechocarpus cauliflorus*

Compound	Anti HSV-1 IC_{50} (μg/ml)	Anti TB MIC (μg/ml)	Cytotoxicity IC_{50} (μg/ml)			
			NCI-H187	KB	BC	Vero cell
Galgravin (SC-1)	moderately active	200	inactive	inactive	inactive	>50
Licarin A (SC-3)	inactive	25	13.67	5.07	4.06	>50
Acuminatin (SC-4)	inactive	12.5	inactive	16.32	inactive	15.8
Engeletin (SC-5)	inactive	50	inactive	ND	ND	>50
Aristolactam AII (SC-8)	moderately active	inactive	inactive	inactive	inactive	ND
Piperolactam D (SC-10)	moderately active	50	inactive	6.43	5.52	7.0
Rifampicin	-	0.0047	-	-	-	-
Kanamycin sulfate	-	2.5	-	-	-	-
Isoniazid	-	0.05	-	-	-	-
Ellipticine	-	-	0.15-0.49	0.21	0.27	-
Doxorubicine	-	-	0.02-0.04	0.14	0.17	-
Acyclovir	0.9-1.9	-	-	-	-	-

ND = not determined

3.1.2 Antituberculosis activity

Nearly all the compounds (except aristolactam AII) isolated from this plant and subjected to this assay were found to be more or less active. Acuminatin exhibited the strongest antimycobacterial activity among the compounds tested (MIC = 12.5 μg/ml). It was about twice more active than licarin A (MIC = 25 μg/ml), indicating the effect of the C-4′ substitution on this activity might be opposite from that observed for cytotoxic activity. Furthermore, engeletin, piperolactam D and galgravin exhibited antituberculosis activity with MIC value of 50, 50 and 200 μg/ml, respectively.

3.1.4 Anti HSV-1 acitivity

Galgravin, the 2,5 diarylfuranoid lignan, showed moderate anti HSV-1 activity, similar to aristolactam AII and piperolactam D. Previously reported antiviral activity of this lignan concerned its ability to suppress hepatitis B virus surface antigen production at the concentration of 25 μM (Huang *et al.*, 2001).

3.1.5 Inhibition of aldose reductase acvitity

Engeletin and astilbin were isolated from fraction G-10 of the EtOAc extract, which was the fraction showing the highest % inhibition of aldose reductase enzyme (at 5 μg/ml). When tested, engeletin and astilbin were able to inhibit the enzyme at 84.9 and 57.8%, respectively. The IC_{50} values of both dihydroflavonol rhamnosides, compared with quercetin as the positive control, are shown in **Table 39**.

Of the three flavonoids, engeletin exhibited the strongest inhibitory activity against AR, with IC_{50} value of 1.16 μM, which was twice that of quercetin. Astilbin, previously isolated from the leaves of *Engelhardtia chrysolepis* and reported as an inhibitor of both rat lens and human recombinant AR (Haraguchi *et al.*, 1997), displayed the activity at IC_{50} value of 26.7 μM, which was about 10 times less active than quercetin and 23 times less active than engeletin. Interestingly, the only structural difference between engeletin and astilbin is the number of hydroxy groups in their C ring, of which only astilbin has the catechol (*ortho*) orientation. And although there have been reports of enhancement of AR inhibitory activity in flavonoids with this partial structure (Varma and Kinoshita, 1976; Okuda *et al.*, 1982), the effect appears to be the opposite for these two glycosides.

Table 39. Inhibitory activity of engeletin and astilbin against AR and AGEs formation.

Compound	IC_{50} (μM)	
	AR	AGEs
Engeletin	1.16	ND
Astilbin	26.7	0.76
Quercetin	2.48	1.01

ND = not determined

3.1.6 Inhibition of AGEs formation

The % inhibition of AGEs formation of engeletin and astilbin (at 2.5 μg/ml) were 13.6 % and 64.9 %, respectively. Only astilbin was then further evaluated for its IC_{50} (**Table 39**).

Astilbin was about as potent as quercetin, which has been reported as a protein glycation inhibitor (Morimitsu *et al.*, 1995; Ahmed, 2005). The presence of 2:3 unsaturation in quercetin or the 3-OH glycosylation by rhamnose in astilbin appears to have no significant effect upon the AGEs formation inhibitory activity of these flavonoids. Inhibitory activity of natural compounds on the Maillard reaction is considered to be closely associated with their antioxidant property (Nagasawa *et al.*, 2003) and both astilbin and its aglycone, taxifolin (2,3-dihydro quercetin), have been demonstrated to protect against oxidative damage (Haraguchi *et al.*, 1996a). Therefore, antioxidant flavonoids such as astilbin and quercetin possess therapeutic potential in the protection and improvement of diabetic complications resulting from the accumulation of advanced glycation end products.

It should also be noted that the number of hydroxy groups in the aglycones of engeletin and astilbin might be a factor in their activities against AR and AGEs formation. Catechol orientation of hydroxy groups in ring C might be better for conferring inhibitory activity on AGEs formation, but not for AR inhibition.