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TETRAHEDRON

Sesquiterpenoids from *Neolitsea parvigemma*: Isolation, Oxidation Products and Antiplatelet Actions

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Abstract. Two novel sesquiterpenoids of the furanogermacrane type, deacetylzeylanine (**9**) and parvigemonol (**10**), have been isolated from the stems of *Neolitsea parvigemma*. The autoxidation of the known pseudoneolinderane (**6**) afforded two unreported sesquiterpenoid dilactones, pseudoneolinderane-A (**11**) and pseudoneolinderane-B (**12**). Some of the sesquiterpenes showed significant inhibitory effects on platelet aggregation. The biogenetic pathway and the structure-activity relationships of some sesquiterpenoids are also discussed.

Keywords: sesquiterpenoid, *Neolitsea parvigemma*, isolation, oxidation, antiplatelet actions

INTRODUCTION

We have previously reported the isolation of eight sesquiterpenoids, parvigemone (**1**), neolitrane (**2**), zeylanidine (**3**), zeylanicine (**4**), linderalactone (**5**), pseudoneolinderane (**6**), linderane (**7**) and deacetylzeylanidine (**8**), and five alkaloids from the stems of *Neolitsea parvigemma* Kan & Sas (Lauraceae).[1,2] In our continuing phytochemical investigation, two new sesquiterpenoids, deacetylzeylanine (**9**) and parvigemonol (**10**), were isolated from the stems of this plant. In order to examine the possible biological activities of compound **6** and its autoxidation products, it was dissolved in $CHCl_3$ and exposed to air at room temperature for 14 days. This afforded two unreported sesquiterpenoid dilactones, pseudoneolinderane-A (**11**) and pseudoneolinderane-B (**12**). Among them, compounds **3**, **4**, **9a** (the acetylation product of **9**)

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and zeylanidine-B (**14**) showed significant inhibition of platelet-activating factor (PAF) induced platelet aggregation. Compounds **5** and **9** showed significant inhibition of arachidonic acid (AA) induced platelet aggregation. Compounds **1**, **9a**, **10**, zeylanidine-A (**13**) and **14** showed significant inhibition of collagen-induced platelet aggregation (**13** and **14** were the autoxidation products of **3**.[3])

RESULTS AND DISCUSSION

Compound **9** was isolated as colorless prisms. The HREIMS showed a $[M]^+$ at *m/z* 260.1045 corresponding to the molecular formula $C_{15}H_{16}O_4$ (calcd 260.1049, Δ-1.5 ppm). An IR absorption band at 1770 cm^{-1}, a λ max at 210 nm in the UV spectrum and signals at δ 131.06 (s), 149.13 (d) and 172.26 (s) in ^{13}C NMR spectrum (Table 1) provided evidence for an α, β-unsaturated γ-lactone.[4] The presence of a trisubstituted-furan moiety was indicated by absorptions at 3100, 1720 and 890 cm^{-1} in the IR spectrum and an unresolved quartet at δ 7.17 in the ^1H NMR spectrum (Table 2) and three singlets (δ 120.43, 146.95 and 120.98) as well as a doublet (δ 139.50) in the ^{13}C NMR spectrum.[4] On comparison of the ^1H and ^{13}C NMR data of the 10-membered macrocyclic moieties of both compounds **9** and zeylanine (**15**) (Tables 1 and 2),[4,5] it was found that a singlet at δ 1.90 (3H) for the acetate group in compound **15** disappeared, while a double doublet at δ 3.94 (J=11.2; 4.6 Hz) for H-2 was observed for compound **9**. The above information along with the signal at δ 68.41 (d) in the ^{13}C NMR spectrum clearly indicated that compound **9** contained a hydroxyl group at C-2 instead of an acetate group as in compound **15**. The inference was confirmed by acetylation of **9** with acetic anhydride to give an acetate (**9a**) which had the same mp, ^1H and ^{13}C NMR characteristics as those of **15**. In order to determine the stereochemistry of the chiral centers at C-2, NOESY experiments were performed. Besides the salient common NOE interactions between the adjacent protons, an interaction between H-2 and H-3a, H-2 and H-4a, were evident. The results of NOESY experiments, coupling patterns, studies of Dreiding models and X-ray crystallography analysis (Fig. 1) indicated that H-2 was assigned with the α-orientation and H-7 with the β-orientation. The above observations and the analysis of its COSY and HETCOR spectra led to establishment of the structure of this compound as **9** (Fig. 2) and the relative configuration of **9** is (2R*, 7S*). Joshi *et al.* had hydrolyzed zeylanine (**15**) to obtain this compound in 1967,[4] but its geometry was not defined. This compound was isolated from

natural sources for the first time and its conformation was further confirmed by X-ray diffraction.

Table 1. ^{13}C NMR chemical shifts of compounds 9, 9a, 10 and 15

Carbon	9[a]	9a[a]	10[a]	15[b]
C-1	145.05 s	137.83 s	151.34 s	139.80 s
C-2	68.41 d	70.99 d	73.43 d	70.46 d
C-3	26.87 t	24.52 t	27.70 t	24.50 t
C-4	21.17 t	20.97 t	20.44 t	20.94 t
C-5	131.06 s	130.61 s	132.15 s	130.57 s
C-6	149.13 d	149.74 t	152.07 d	149.73 d
C-7	73.97 d	74.10 t	73.83 d	74.07 d
C-8	120.43 s	120.38 s	115.82 s	120.36 s
C-9	146.95 s	146.95 s	153.59 s	146.93 s
C-10	119.32 d	122.32 d	115.60 t	122.28 d
C-11	120.98 s	120.67 s	120.73 s	120.75 s
C-12	139.50 d	139.69 d	138.48 d	139.65 d
C-13	8.03 q	8.12 q	7.79 q	8.09 q
C-14	17.05 q	17.64 q	18.32 q	17.60 q
C-15	172.26 s	172.13 s	174.03 s	172.08 s
Ac		20.88 q		20.86 q
Ac		168.88 s		168.83 s

[a]Measured at 50 MHz, in $CDCl_3$, with TMS as internal standard. Chemical shifts are in δ values.

[b]Measured at 75 MHz, in $CDCl_3$, with TMS as internal standard. Chemical shifts are in δ values.[5]

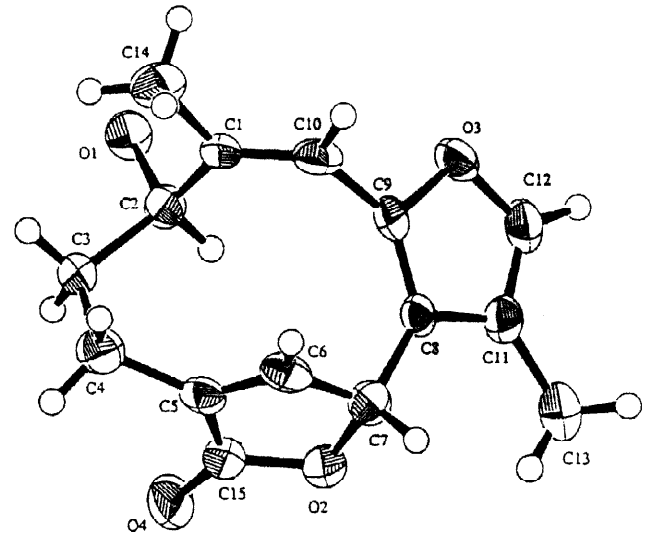


Figure 1 Molecular structure (relative configuration) of deacetylzeylanine (9)

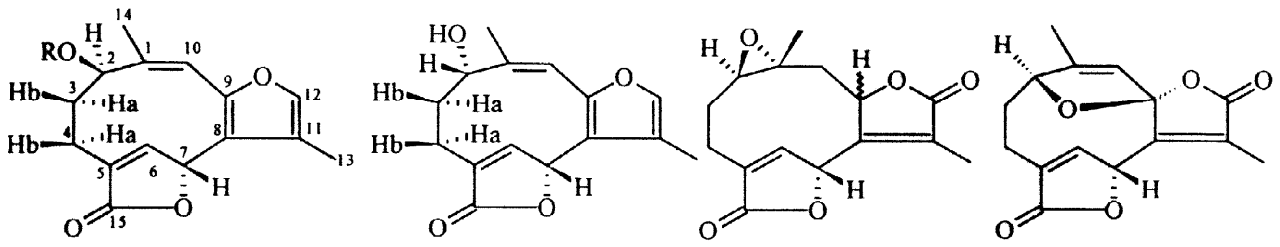
Compound **10** was isolated as colorless prisms and had the molecular formula $C_{15}H_{16}O_4$ from the molecular ion at *m/z* 260.1053 in HREIMS (calcd 260.1049, Δ+1.5 ppm). Based on ^{13}C and ^{1}H NMR spectral analysis (Tables 1 and 2), the structural features in the furanogermacrane moiety of **10** are very close to those of compound **9**. An IR absorption band at 1760 cm^{-1}, a λ max at 212 nm in the UV spectrum, and signals at δ 132.15 (s), 152.07 (d) and 174.03 (s) in ^{13}C NMR spectrum provided evidence for an α, β-unsaturated γ-lactone.[4] The presence of a furan ring was revealed from the IR (3100, 1710 and 900 cm^{-1}), ^{1}H NMR

Table 2. ^{1}H NMR chemical shifts of compounds **9**, **9a**, **10** and **15**

Proton	9[a]	9a[a]	10[a]	15[b]
H-2	3.94 dd (11.2;4.6)	4.91 dd (12.0; 5.4)	4.46 d (5.0)	
H-3a	1.73-1.80 m	2.07 d, (6.0)	1.81-1.95 m	
H-3b	2.24-2.28 m	2.24 m	2.38-2.46 m	
H-4a	2.29-2.34 m	2.35 m	2.47-2.55 m	
H-4b	2.64-2.67 m	2.70 m	3.07-3.14 m	
H-6	7.07 d (1.8)	7.11 s	6.83 d (2.0)	7.10 m
H-7	5.72 d (1.8)	5.75 s	5.76 d (2.0)	6.22 m
H-10	6.14 s	6.25 s	6.19 s	
H-12	7.17 q (unresolved)	7.24 s	7.19 s	7.20 d
H-13	2.12 s	2.13 s	2.13 s	2.12 d (1.1)
H-14	1.93 s	1.90 s	1.49 s	1.88 s
OAc		1.92 s		1.90 s

[a] Measured at 200 MHz, in $CDCl_3$, with TMS as internal standard. Coupling constants (J in Hz) are in parentheses. Chemical shifts are in δ values.
[b] Measured at 60 MHz.[4]

(δ 7.19, s) and ^{13}C NMR (δ 115.82 s, 153.59 s, 120.73 s and 138.48 d). On comparison of the ^{1}H and ^{13}C NMR data of the 10-membered macrocyclic moieties of both compounds **9** and **10**, it was found that a double doublet at δ 3.94 for H-2 in compound **9** disappeared, while a doublet at δ 4.46 (J = 5.0 Hz) for H-2 was observed for compound **10**. The above information along with signal at δ 73.43 (d) in the ^{13}C NMR spectrum clearly indicated that compound **10** also contained a hydroxyl group at C-2. The chemical shifts at δ 5.76 for H-7 and δ 73.83 for C-7 in **10** were close to those of **9**, therefore, the stereochemistry of C-7 in **10** was assigned as β-orientation, the same as that of **9**. In order to determine the stereochemistry of the chiral center at C-2, NOESY experiments were performed. Besides the salient common NOE interactions between the adjacent protons, an interaction between H-2 and H-3b, H-2 and H-4b were evident. Based on the results of NOESY experiments, coupling patterns, studies of Dreiding models, and compared with the structure of **9**, H-2 was assigned with the β-orientation. The above observations and the analysis of its COSY and HETCOR spectra led to the establishment of the structure of this compound as **10** (Fig. 2). Compounds **9** and **10** are diastereoisomers.



9 R=H
9a (15) R=Ac

10 **11** **12**

Figure 2 The structure of compounds **9**, **9a**, **10** **11** and **12**

Compound **11** was obtained as white needles. Its molecular formula was established as $C_{15}H_{16}O_5$ by HREIMS (found: 276.1051, calcd: 276.1056, Δ-1.8 ppm). The UV spectrum showed maxima at 226 nm being characteristic of two unsaturated lactones.[6] The IR spectrum showed the presence of two unsaturated lactones (1755 and 1780 cm^{-1}). Two lactone functionalities were supported by signals at δ 171.29 and 173.64 in the ^{13}C NMR spectrum (Table 3). Since this compound is an autoxidation product, the additional lactone function must have resulted from oxidation of the furan moiety. The fact that the signal at δ 7.12 for H-12 in **6** was absent and replaced by a new multiplet at δ 5.19-5.22 for H-9 in **11** (Table 4) strongly indicated oxidation of the furan moiety. The chemical shifts of **11** were compared with those of the starting material **6**. Of the two H-10 protons in **11**, one was shifted more upfield (δ 2.35) and the other more downfield (δ 3.33), relative to those of the corresponding protons (δ 2.60-2.73) in the parent compound **6**. The remaining signals in the ^{1}H NMR spectrum, δ 0.96 for H-14, δ 2.02 for H-13, δ 7.04 for H-6 and δ 6.11 for H-7, were close to those of **6**. The above observations and the analysis of its COSY spectrum led to the establishment of the structure of this compound as **11** (Fig. 2).

Compound **12** was obtained as white needles. Its molecular formula was established as $C_{15}H_{14}O_5$ by HREIMS (found: 274.1043, calcd: 274.1046, Δ-1.1 ppm). The presence of two unsaturated lactones was indicated by a UV absorption at 228 nm, IR bands at 1790 and 1760 cm^{-1} and ^{13}C NMR signals at δ 174.33, 169.18, 147.95 and 131.91 (Table 3). The ^{1}H NMR

spectrum (Table 4) showed two vinyl methyl groups at δ 1.89 (3 H, s) and 2.04 (3 H, s) attached to C-1 and C-11, respectively. On comparison of the ^{13}C and ^{1}H NMR data of the 10-membered macrocyclic moieties of both compounds **12** and neoliacine **16** (Tables 3 and 4),[3,7] it was found that a singlet at δ 4.31 for H-6 in compound **16** disappeared, while another singlet at δ 7.21 for H-6 was observed in compound **12**. The above information along with signals at δ 129.20 (s) and 148.90 (d) in the ^{13}C NMR spectrum clearly indicated that compound **12** contained a double bond at C-5 through C-6 instead of an epoxy function between C-5 and C-6 as in compound **16**. The above observations and the analysis of its COSY spectrum led to establishment of the structure of this compound as **12** (Fig. 2).

Table 3. ^{13}C NMR chemical shifts of compounds **6**, **11**, **12**, and **16**

Carbon	6	11	12	16[3,7]
C-1	58.64 s	57.70 s	146.81 s	147.3 s
C-2	65.71 d	65.43 d	89.30 d	88.1 d
C-3	23.85 t	25.46 t	29.69 t	27.1 t
C-4	18.68 t	19.83 t	18.82 t	18.4 t
C-5	131.48 s	128.91 s	129.20 s	55.5 s
C-6	147.31 d	149.33 d	148.90 d	60.3 d
C-7	74.31 d	74.27 d	74.28 d	71.1 d
C-8	115.29 s	154.85 s	147.95 s	147.9 s
C-9	150.38 s	78.47 d	115.88 s	115.1 s
C-10	37.40 t	38.72 t	121.65 d	122.8 d
C-11	120.80 s	130.30 s	131.91 s	132.9 s
C-12	137.31 d	171.29 s	169.18 s	169.4 s
C-13	8.36 q	9.29 q	8.82 q	8.8 q
C-14	16.36 q	16.81 q	12.76 q	12.2 q
C-15	1.94 s	173.64 s	174.33 s	172.2 s

Measured at 50 MHz, in $CDCl_3$, with TMS as internal standard.
Chemical shifts are in δ values.

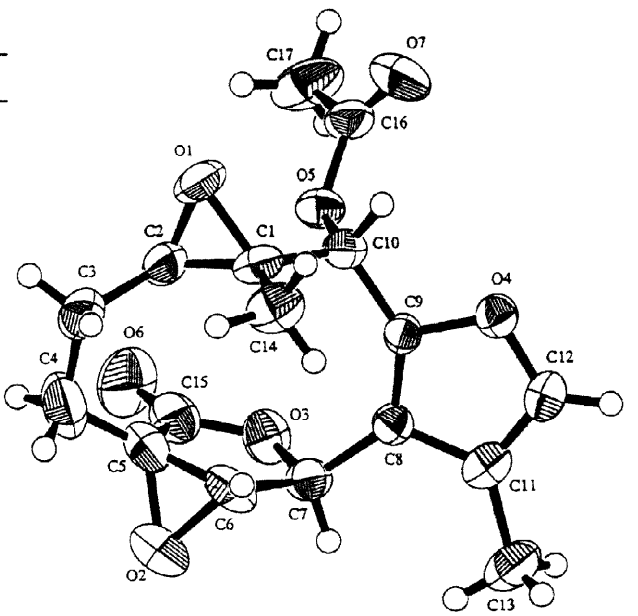


Figure 3 Molecular structure (relative configuration) of zeylanidine (3)

Compound **3** was first isolated from the roots of *Neolitsa zeylanica*[8] and the stereochemistry at C-1, C-2 and C-10 was reported by W. S. Lee *et al.*[9] In order to determine the stereochemistry at the other chiral centers of this compound, an X-ray diffraction analysis

Table 4. ^{1}H NMR chemical shifts of compounds 6, 11, 12 and 16

Proton	6	11	12	16[3,7]
H-2	2.79 dd (11.7; 2.0)	2.78-2.91 m	5.10 d (3.2)	5.16 br.s
H-3	2.24-2.26 m	1.79-1.90 m	2.05-2.10 m	2.06 m
H-3	2.57-2.61 m	2.19-2.35 m	2.44-2.60 m	2.20 m
H-4	1.50-1.54 m	1.51-1.59 m	2.44-2.60 m	1.83 m
H-4	2.76-2.79 m	2.74-2.77 m	2.44-2.60 m	2.55 ddd (15.8; 11.5; 6.5)
H-6	7.05 s	7.04 s	7.21 s	4.31 s
H-7	5.88 s	6.11 s	5.92 s	5.41 s
H-9		5.19-5.22 m		
H-10	2.35 d (15.4)	2.60-2.73 m	5.27 s	5.32 s
H-10	3.33 d (15.4)			
H-11	7.12 d (1.1)			
H-13	2.08 d (1.1)	2.02 s	2.04 s	2.00 s
H-14	0.99 s	0.96 s	1.89 s	1.89 d (1.0)

Measured at 200 MHz, in $CDCl_3$, with TMS as internal standard.
Coupling constants (J in Hz) are in parentheses.
Chemical shifts are in δ values.

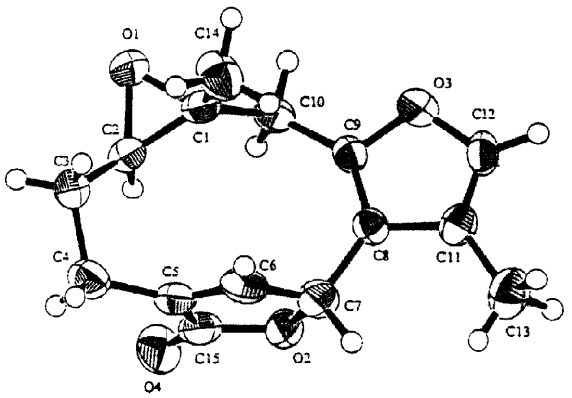
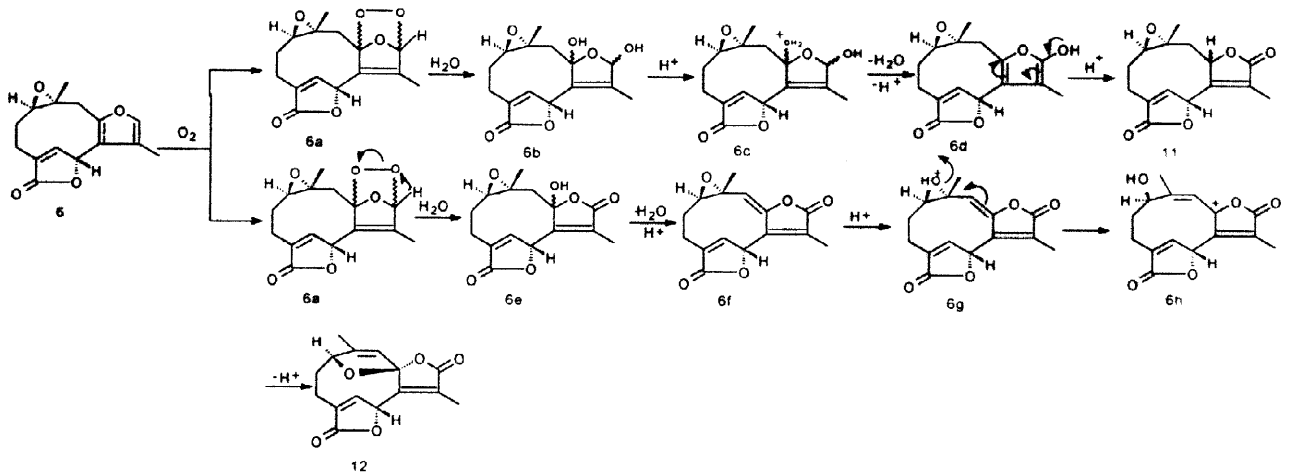


Figure 4 Molecular structure (relative configuration) of pseudoneolinderane (6)

of a single crystal was thus undertaken. The X-ray structure (Fig. 3) indicated that the relative configuration of **3** is (1S*, 2R*, 5S*, 6R*, 7R*, 10S*).

The stereochemistry at C-1, C-2 and C-7 in compound **6** has not been reported previously. In order to determine the stereochemistry of these chiral centers in the compound, X-ray experiments were performed. The X-ray analysis (Fig. 4) showed that the relative configuration of **6** is (1R*, 2R*, 7R*).

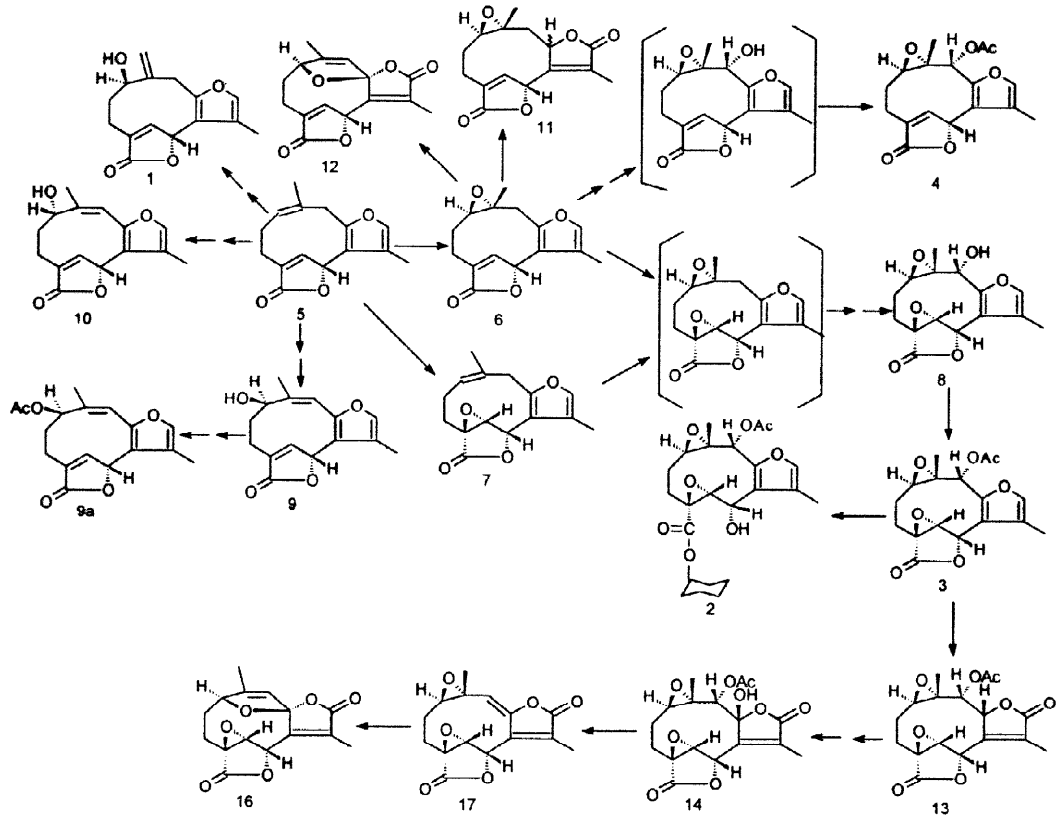
A possible mechanism for the oxidation of **6** with O_2 in which **11** and **12** are formed is summarized in Scheme 1. The reaction of **6** with O_2 gave peroxide **6a,**[10,11] which was hydrolyzed to **6b** by the treatment of water. Protonation of **6b** and subsequent loss of water and a proton lead to a stable aromatic furan moiety **6d** which could then tautomerize to a more stable compound **11. 6a** might be reduced to **6e** by the treatment of water. Dehydration of **6e** and subsequent protonation lead to **6g** from which subsequent ring opening would form carbocation **6h.** Finally, **12** was generated by elimination of a proton from **6h.**



Scheme 1 A possible mechanism for the autoxidation of **6**

From the stems of *Neolitsea parvigemma*, we obtained a series of sesquiterpenoid compounds. Based on these structural similarities, it was tempting to propose a biogenetic pathway which interrelates these sesquiterpenoids. A reasonable premise, following from the putative biogenetic scheme outline in Scheme 2, is that compound **5** is the precursor of the furanosesquiterpenoid skeleton. Appropriate functionalization or epoxidation of **5**, could be considered to be the compounds **1**, **6**, **7**, **9** or **10**. Acetylation of **9** gives **9a**. Furan ring oxidation or functionalization of **6** would lead directly to **11**, **12** or a closely related structures **4** and **8**. Epoxidation and subsequent hydroxylation of **7** can also give **8**. Acetylation of **8** gives **3**. Compound **3**, after opening of the lactone ring with attachment of cyclohexanol, would give **2**; oxidation of the furan ring would yield **13**. Hydroxylation of **13** affords **14** which could form **17**. **16** can be generated by protonation and a subsequent ring opening of **17**. Compounds **16** and **17** were the autoxidation products of **3**.[3]

Compounds **1-9, 9a, 10, 13** and **14** were screened for platelet aggregation. The results are shown in Table 5. As indicated in Table 5, at a concentration of 100 μ g/ml, compounds **3**, **4**, **9a** and **14** showed significant inhibition of platelet-activating factor (PAF) induced platelet aggregation. Compound **5** showed strong inhibition and **9** showed significant inhibition of arachidonic acid (AA) induced platelet aggregation. Compounds **1, 9a, 10, 13** and **14** showed significant inhibition of collagen-induced platelet aggregation. From the results obtained, the



Scheme 2 A possible biogenetic scheme of sesquiterpenoid compounds

following four conclusions can be drawn in terms of antiplatelet effects. First, the germacrane-type furanosesquiterpenoids containing two double bonds at C-1 and C-5, for example **5**, showed strong inhibition of platelet aggregation caused by AA, but when the two double bonds were converted into an epoxide ring at C-1 through C-2 or at C-5 through C-6, or acetyl or hydroxyl at C-2, for example, **3**, **9a** and **10**, the antiplatelet effects were reduced. Second, the collagen antiplatelet effects of the germacranodilactones, for example **13** and **14**, were more potent than those of the germacranolactones among the test compounds. Third, germacrane-type furanosesquiterpenoids containing an acetyl group at C-2, for example **9a**, showed significant inhibition of platelet aggregation caused by PAF, but when the acetyl group was converted into an hydroxyl group, for example **1** and **9**, the antiplatelet effects were reduced. Finally, all the test compounds were not active for the antiplatelet aggregation caused by ADP.

Table 5. Effects of tested compounds on the platelet aggregation induced by ADP, AA, collagen and PAF in washed rabbit platelets.

	Aggregation (%)			
Compound	ADP(20 μM)	AA(100 μM)	Col.(10 μg/ml)	PAF(2ng/ml)
control	92.7±0.8	85.7±2.3	85.1±1.2	89.1±1.1
1(100 μg/ml)	87.6±3.6	81.7±2.0	59.2±7.1*	64.3±2.9
2(100 μg/ml)	88.6±1.7	72.5±7.0	81.1±1.9	82.6±2.2
3(100 μg/ml)	90.5±0.9	82.4±1.7	81.5±1.1	55.2±9.5*
4(100 μg/ml)	90.9±0.3	82.0±1.7	81.5±1.4	50.0±10.9*
5(100 μg/ml)	92.2±2.6	25.2±4.5***	88.0±4.0	71.2±4.6
6(100 μg/ml)	90.6±1.7	81.5±1.2	84.7±4.3	82.8±0.5
7(100 μg/ml)	83.7±1.0	77.8±1.2	75.4±4.6	77.5±1.4
8(100 μg/ml)	89.5±1.0	82.2±1.6	80.9±2.0	84.9±1.0
9(100 μg/ml)	88.7±2.9	58.3±12.8*	63.5±15.9	78.2±1.9
9a(100 μg/ml)	87.4±3.4	61.9±9.4	34.4±17.5**	44.8±6.5**
10(100 μg/ml)	88.5±2.7	72.3±3.1	55.7±16.1*	72.1±2.3
13(100 μg/ml)	84.9±1.0	65.3±5.6	31.8±12.4**	68.6±2.1
14(100 μg/ml)	83.6±1.4	66.9±3.9	31.0±5.8**	59.2±3.2*
Aspirin(25 μg/ml)	77.9±1.9	0.0±0.0	87.8±1.5	90.4±1.1

Platelet were preincubated with DMSO (0.5 %, control), aspirin or tested compounds at 37 ℃ for 3 min, t then ADP (20 μ M), AA (100 μ M), collagen (col., 10 μ g / ml) or PAF (2 ng/ml) was add. Percentage of aggregation are presented as means ± S.E. (n = 3 ~ 5). * P < 0.05, ** P< 0.01,*** P< 0.001 as compared with the respective control.

EXPERIMENTAL

General. Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR spectra were recorded with a Varian NMR spectrometer at 200 MHz (Gemini) and ^{13}C NMR spectra were recorded with a Varian Gemini NMR spectrometer at 50 MHz, in $CDCl_3$ using TMS as internal standard. EIMS were obtained with a JEOL JMS-HX110 mass spectrometer at 70 eV. A Rigaku AFC 6S diffractometer was used in the X-Ray work. Silica gel 60 (Merck, 230-400 mesh) was used for column chromatography, precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC, and precoated silica gel plates (Merck,

Kieselgel 60 F-254, 0.50 mm) were used for prep. TLC.

Plant Material. The stems of *Neolitsea parvigemma* were collected from Pingtung-Hsien, Taiwan in June, 1992, identified by Dr. Feng-Chi Ho. A voucher specimen was deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. Air-dried, powdered stems (2.62 kg) of *N. parvigemma* were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and partitioned to yield $CHCl_3$- and H_2O- soluble extracts. A portion of the $CHCl_3$ soluble extract (41.0 g) was chromatographed over Si gel (1500 g) and eluted with n-hexane-$CHCl_3$ mixtures of increasing polarities to yield 45 fractions (120 mL each). The fractions (736 mg) eluted from *n*-hexane-$CHCl_3$ (1:1) was rechromatographed on silica gel [*n*-hexane-$CHCl_3$ (1:2)] to yield **9** and **10**.

Deacetylzeylanine (**9**) was obtained as colorless prisms (31 mg) (MeOH): mp 216-217 ℃ ; $[\alpha]_D^{24}$ -185 (*c* 0.48, $CHCl_3$); UV (EtOH) λ max (log ε) nm 210 (4.03); IR (KBr) ν max 3400, 3100, 1770, 1720, 890 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 50 MHz) see Table 1 ; EIMS *m/z* (rel. int.) 260 $[M]^+$ (17), 232 (8), 203 (22), 175 (25), 159 (100), 145 (38), 128 (26), 115 (28); HREIMS *m/z* 260.1045 (calcd for $C_{15}H_{16}O_4$, 260.1049, Δ-1.5 ppm).

Acetylation of deacetylzeylanine (**9a**) was obtained as colorless prisms (12 mg) (MeOH): mp 178-180 ℃ ; $[\alpha]_D^{24}$ -176 (*c* 0.45, $CHCl_3$); UV (EtOH) λ max (log ε) 212 (3.98) nm; IR (KBr) ν max 3100, 1755, 1740, 1245, 895 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m/z* (rel. int.) 302 $[M]^+$ (12), 260 (10), 242 (27), 214 (25), 185 (98), 171 (30), 159 (67), 128 (40), 119 (100).

Parvigemonol (**10**) was obtained as colorless prisms (19 mg) (MeOH) : mp 223-224 ℃;

$[\alpha]_D^{24}$ +135 (*c* 0.32, $CHCl_3$); UV (EtOH) λ max (log ε) 212 (3.96) nm; IR (KBr) ν max 3450, 3100, 1760, 1710, 900 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 1 ; EIMS *m/z* (rel. int.) 260 $[M]^+$ (19), 232 (10), 203 (19), 172 (19), 159 (100), 145 (31), 128 (25), 115 (31); HREIMS *m/z* 260.1053 (calcd for $C_{15}H_{16}O_4$, 260.1049, Δ+1.5 ppm).

Air-oxidation of linderalactone (**5**). The furan rings of the compounds were very susceptible to autoxidation when the compounds were dissolved in solvents such as $CHCl_3$ and exposure to air.[7,12,13] The oxidation followed the procedures described by Ulublen *et al.*[14,15] Compound **5** (63 mg) was dissolved in $CHCl_3$ (20 ml) and left at room temperature for 14 days, by which time all the compound **5** had disappeared. Removal of $CHCl_3$ *in vacuo* left a light brown viscous residue (32 mg) that was chromatographed over silica gel using *n*-hexane-$CHCl_3$ (1:3) to give 3 fractions (I-III). Compound **6** was separated from fraction II and was identical in mp, ^1H NMR and ^{13}C NMR to the isolated compound.

Air-oxidation of pseudoneolinderane (**6**). Compound **6** (500 mg) was dissolved in $CHCl_3$ (100 ml) and left at room temperature for 14 days, by which time all the compound **6** had disappeared. Removal of $CHCl_3$ *in vacuo* left a light-brown viscous residue (72 mg) that was chromatographed over silica gel using *n*-hexane-$CHCl_3$ mixtures of increasing polarity, to yield 6 fractions. The fractions eluted from *n*-hexane-$CHCl_3$ (1:2) were further purified by silica gel column chromatography using the same solvent system to obtain **11**. The fractions eluted from *n*-hexane-$CHCl_3$ (1:4) were further purified by silica gel column chromatography using *n*-hexane- $CHCl_3$ (1:3) solvent to obtain**12**.

Pseudoneolinderane-A (**11**) was obtained as white needles (14 mg) (MeOH): mp 215-217 ℃; $[\alpha]_D^{24}$ +240 (*c* 0.80, $CHCl_3$); UV (EtOH) λ max (log ε) 226 (4.06) nm; IR (KBr) ν max 1780, 1755 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 3; EIMS *m/z* (rel. int.) 276 $[M]^+$ (18), 258 (39), 230 (39), 205 (31), 187 (37), 160 (100), 123 (57); HREIMS *m/z* 276.1051 (calcd for $C_{15}H_{16}O_5$, 276.1056, Δ-1.8 ppm).

Pseudoneolinderane-B **(12)** was obtained as white needles (10 mg) (MeOH): mp 274-276 ℃; $[\alpha]_D^{24}$ +220 (*c* 0.80, $CHCl_3$); UV (EtOH) λ max (log ε) 228 (3.86) nm; IR (KBr) ν max 1790, 1760 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 3; EIMS *m/z* (rel. int.) 274 $[M]^+$ (27), 246 (13), 200 (16), 174 (96), 133 (100), 115 (24); HREIMS *m/z* 274.1043 (calcd for $C_{15}H_{14}O_5$, 274.1046, Δ-1.1 ppm).

X-ray Structure Determination. Crystals [**3, 6** and **9**] for diffraction study were all obtained from MeOH- $CHCl_3$ solvent mixtures. Structures were solved via direct method (SIR92)[16] and refined with a full- matrix least-squares program using the teXsan[17] software package. Anisotropic refinement was carried out for all non-hydrogen atoms. Hydrogen atoms were calculated according to their idealized positions (d_{C-H}=0.95A°) but not refined. The hydroxyl proton on O (1) in **9** was not located. The absolute configuration for all three compounds was not determined due to lack of strong scattering atoms. Full crystallographic data has been deposited at Cambridge Crystallographic Data Centre and in available as supplementary information.

Biological Assay. Platelet aggregation testing carried out according to ref.[18,19]

Data Analysis. The experimental results are expressed as means ± S.E. and accompanied by the number of observations. A one-way analysis of variance (ANOVA) was used for multiple comparison, and if there was significant variation between treatment groups, then the mean values for inhibitors were compared with those for controls by the Student's *t* test, and *p* values of less than 0.05 were considered to be statistically significant[20]

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