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Secondary metabolites from the leaves of *Neolitsea hiiranensis* and the anti-inflammatory activity of some of them

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ABSTRACT

Seven sesquiterpenoids, hiiranlactones A–D (**1**–**4**), (−)-*ent*-6α-methoxyeudesm-4(15)-en-1β-ol (**5**), (+)-villosine (**6**), hiiranepoxide (**7**), and one triterpenoid, hiiranterpenone (**8**), together with 22 known compounds, were isolated from the leaves of *Neolitsea hiiranensis* (Lauraceae). Their structures were elucidated by spectroscopic analysis and single crystal X-ray diffraction. Among the isolates, hiiranlactone B (**2**) and hiiranlactone D (**4**) exhibited inhibitory activity against fMLP-induced superoxide production by human neutrophils with IC_{50} values of 21.86 ± 3.97 and 25.78 ± 4.77 μM, respectively.

1. Introduction

Neolitsea hiiranensis Liu and Liao (Lauraceae) is a small evergreen tree endemic to Taiwan and found only in the Hengchun Peninsula (Liao, 1996). Neolitsea plants have been found to be rich in sesquiterpenoids (Takaoka et al., 1993a), triterpenoids (Yano et al., 1992), alkaloids (Lee et al., 2007), and steroids (Yano et al., 1992). The occurrence of a few flavonoids (Lam et al., 2008), benzenoids (Chang et al., 2002), monoterpenoids (Ouyang et al., 2007), and benzoquinone (Chang et al., 2002) have also been reported. Analysis of the chemical constituents of the leaves of *N. hiiranensis* has not yet been conducted, except for nine sesquiterpenoids, one triterpenoid and two steroids which were obtained from roots of this plant (Wu and Li, 1995). Investigation of the ethyl acetate-soluble layer of the leaves of this species led to the isolation of eight new compounds (**1**–**8**) (Fig. 1), along with 22 known compounds. The structure elucidations of **1**–**8** were based on spectroscopic analysis and single crystal X-ray diffraction. This paper describes the structure elucidation compounds of **1**–**8**.

2. Results and discussion

Hiiranlactone A (**1**) was obtained as optically active colorless needles with $[\alpha]_D^{25}$ −17.6 (*c* 0.06, $CHCl_3$). Its molecular formula was established as $C_{17}H_{22}O_5$ by HRESIMS, with 7°. Analysis of the unsaturation IR and ^{13}C NMR spectra showed absorptions at 1766 cm^{-1} for a lactone carbonyl group (δ 171.5, C-12) and at 1719 cm^{-1} for a conjugated carbonyl group (δ 167.9, C-15; 140.9, C-5; 125.5, C-4). The ^1H NMR spectrum (Table 1) displayed two exomethylenes [δ 5.52 (1H, *s*, H_a-4) and 6.30 (1H, *s*, H_b-4); 4.89 (1H, *d*, *J* = 17.4 Hz, H_a-3) and 4.90 (1H, *d*, *J* = 11.2 Hz, H_b-3)], an olefinic proton [δ 5.58 (1H, *dd*, *J* = 17.4, 11.2 Hz, H-2)], a tertiary methyl group [δ 1.13 (3H, *s*, H-14)], a vinylic methyl group [δ 1.88 (3H, *s*, H-13)], a methine proton [δ 2.90 (1H, *dd*, *J* = 9.4, 7.4 Hz, H-6)], two methylenes [δ 1.69 (1H, *d*, *J* = 13.8 Hz, H_a-10) and 2.25 (1H, *d*, *J* = 13.8 Hz, H_b-10); 2.55 (1H, *br d*, *J* = 9.4 Hz, H_a-7) and 2.56 (1H, *br d*, *J* = 7.4 Hz, H_b-7)], a carboxymethyl group [δ 3.70 (3H, *s*, H-16)], and a methoxy group [δ 3.18 (3H, *s*, H-17)] on an acetal carbon (δ 105.5, C-9) in the ^{13}C NMR spectrum suggesting the presence of an elemane type sesqutierpenoid (Fu et al., 2001). The NMR spectrum of **1** was similar to that of edwarsolide B (Bifulco et al., 1993), except that the methoxy group (δ_H 3.18) on the acetal at C-9 (δ 105.5) in **1** was replaced by a hydroxy group on

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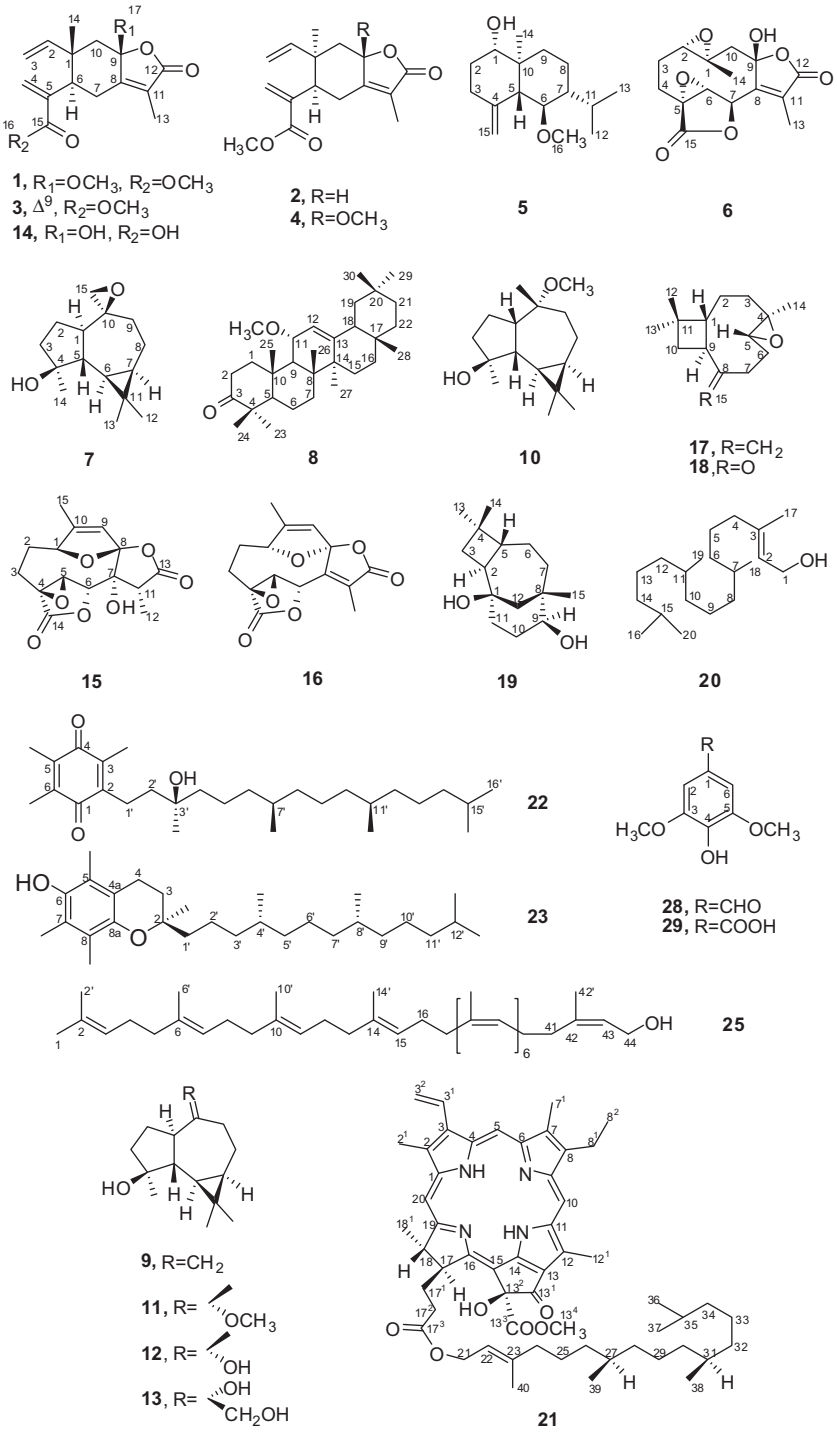

Fig. 1. Structures of compounds **1**–**23**, **25**, **28**–**29**.

the hemiacetal C-9 (δ 104.7) of edwardsolide B. The NOESY spectrum showed correlations (Fig. 2) between H-14 (δ 1.13) and the methoxy group (δ 3.18), but no correlation between H-14 (δ 1.13) and H-6 (δ 2.90), suggesting that H-14 and the methoxy group were on the same side of the molecule, and that H-6 was on the opposite side. Thus, the relative configuration of **1** was the same as edwardsolide B. Based on the above evidence, the structure of **1**, named hiiranlactone A, was elucidated as methyl 2-[(5*R**,6*S**,7a*S**)-7a-methoxy-3,6-dimethyl-2-oxo-6-vinyl-2,4,5,6,7,7a-hexahydrobenzofuran-5-yl]acrylate, which was further confirmed by DEPT, HSQC, NOESY (Fig. 2) and HMBC (Fig. 3) experiments.

Hiiranlactone B (**2**) was isolated as optically colorless needles with $[\alpha]_D^{25}$ −22.2 (*c* 0.03, $CHCl_3$). The HRESIMS analysis of **2** gave a quasi-molecular ion peak at *m*/*z* 299.1261 $[M+Na]^+$ (calcd for $C_{16}H_{20}O_4Na$ 299.1259), consistent with a molecular formula of $C_{16}H_{20}O_4$ with 7° of unsaturation. Analysis of the IR, ^1H (Table 1), and ^{13}C NMR (Table 2) spectra indicated the presence of an elemane type sesquiterpenoid (Fu et al., 2001) similar to **1**, except that an oxymethine proton [δ 4.89 (1H, *dd*, *J* = 12.6, 6.0 Hz, H-9); δ_C 78.1] in C-9 of **2** replaced a methoxy group (δ_H 3.18) on the C-9 acetal (δ_C 105.5) of **1**, resulting in the methyl group [δ 1.13 (3H, *s*, H-14)] in **1** being upshifted to δ_H 0.90 in **2**. The relative configuration of **2** was