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COUMARINS AND ANTI-PLATELET AGGREGATION CONSTITUENTS FROM *ZANTHOXYLUM SCHINIFOLIUM*

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Key Word Index—*Zanthoxylum schinifolium*; Rutaceae; bark; coumarins; terpenyl coumarins; alkaloids; anti-platelet aggregation.

Abstract—Six new coumarins, schinicoumarin, acetoxyaurapten, epoxycollinin, schininallylol, schinilenol and schinindiol, along with seven known coumarins, aurapten, collinin, epoxyaurapten, hydrangetin, umbelliferone, acetoxycollinin and aesculetin dimethyl ether, three known alkaloids, norchelerythrine, dictamnine and skimmianine, and two triterpenoids, β-amyrin and friedelin, were isolated and characterized from the chloroform-soluble part of the bark of *Zanthoxylum schinifolium*. The structures of these compounds were elucidated by spectral analyses. Separation accompanied by bioassay-guided fractionation resulted in the isolation of seven compounds with strong inhibitory activity on platelet aggregation *in vitro*. These are schinicoumarin, acetoxyaurapten, schininallylol, aurapten, collinin, (−)-acetoxycollinin and dictamnine.

INTRODUCTION

Zanthoxylum schinifolium is a prickly shrub, distributed in China, Korea, Japan and Taiwan [1]. Its ripe pericarp is one of the sources of Pericarpium Zanthoxyli in China [2]. Chemical constituents of this species, especially from parts of the fruit, have been studied extensively [3–18]. A methanol extract of the bark of the Formosan species showed strong anti-platelet aggregation activity, from which chelerythrine, an anti-platelet agent [19], was detected by TLC. These findings led us to reinvestigate the chemical constituents and anti-platelet principles from *Z. schinifolium*. Examination of the chloroform-soluble part of the bark extract has led to the isolation of six new coumarins (**1–6**), seven known coumarins, three known alkaloids and two known triterpenoids. The known compounds, aurapten (**7**) [20], collinin (**8**) [21], epoxyaurapten (**9**) [22], hydrangetin (**10**) [23], umbelliferone (**11**) [20], (−)-acetoxycollinin (**12**) [15], aesculetin dimethyl ether (**13**) [24], norchelerythrine (**14**) [24], dictamnine (**15**) [24], skimmianine (**16**) [24], β-amyrin (**17**) [25] and friedelin (**18**) [26] were identified by comparison with authentic samples or literature data. In this paper, we also report the structural elucidation of the six new compounds, which include five terpenyl coumarins.

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RESULTS AND DISCUSSION

Schinicoumarin (**1**) was isolated as needles and the molecular formula of $C_{12}H_{12}O_5$ was established by HR and EI-mass spectrometry ($[M]^+$, *m/z* 236). UV absorption at 240, 260 sh) and 318 nm indicated the presence of a coumarin moiety. The IR spectrum exhibited a lactonic carbonyl absorption at 1730 cm^{-1}. The ^1H NMR spectrum of **1** showed two mutual *ortho*-coupling signals at δ7.18 and 6.87 (each 1 H, *d*, *J* = 8.8 Hz) assigned to H-5 and H-6, respectively. The presence of three methoxyl singlets at δ3.89, 3.93, 3.99 and an aromatic proton singlet at δ6.78 suggested 3,7,8-trimethoxycoumarin or 4,7,8-trimethoxycoumarin as the possible structures for **1**. The structure of schinicoumarin was elucidated as 3,7,8-trimethoxycoumarin by comparison with the ^1H NMR spectrum of 3-methoxycoumarin and 4-methoxycoumarin [27]; it was further confirmed by NOESY (Fig. 1), HETCOR, DEPT and ^{13}C NMR experiments.

Acetoxyaurapten (**2**) was isolated as prisms. Its UV spectrum showed characteristic absorptions at 250 sh and 324 nm of a 7-oxygenated coumarin. The IR spectrum indicated the presence of an acetoxy group at 1740 cm^{-1} and a lactonic carbonyl group at 1722 cm^{-1}. In the ^1H NMR spectrum (Table 1), most of the signals were similar to those of aurapten (**7**). But the presence of an acetoxy group [δ1.99(3H, s)] and a methine proton

Table 1. ^{1}H NMR data for several 7-terpenyl coumarins (200 MHz, in $CDCl_3$)

H	7	8	2	12	9	3	5	4	6
3	6.25 (1H, *d*, *J* = 9.4 Hz)	6.26 (1H, *d*, *J* = 9.5 Hz)	6.25 (1H, *d*, *J* = 9.4 Hz)	6.27 (1H, *d*, *J* = 9.5 Hz)	6.26 (1H, *d*, *J* = 9.5 Hz)	6.27 (1H, *d*, *J* = 9.6 Hz)	6.27 (1H, *d*, *J* = 9.5 Hz)	6.27 (1H, *d*, *J* = 9.5 Hz)	6.27 (1H, *d*, *J* = 9.5 Hz)
4	7.65 (1H, *d*, *J* = 9.4 Hz)	7.63 (1H, *d*, *J* = 9.5 Hz)	7.64 (1H, *d*, *J* = 9.4 Hz)	7.63 (1H, *d*, *J* = 9.5 Hz)	7.64 (1H, *d*, *J* = 9.5 Hz)	7.63 (1H, *d*, *J* = 9.6 Hz)	7.63 (1H, *d*, *J* = 9.5 Hz)	7.63 (1H, *d*, *J* = 9.5 Hz)	7.64 (1H, *d*, *J* = 9.5 Hz)
5	7.37 (1H, *d*, *J* = 8.3 Hz)	7.15 (1H, *d*, *J* = 8.8 Hz)	7.37 (1H, *d*, *J* = 8.2 Hz)	7.15 (1H, *d*, *J* = 8.6 Hz)	7.37 (1H, *d*, *J* = 8.1 Hz)	7.15 (1H, *d*, *J* = 8.7 Hz)	7.16 (1H, *d*, *J* = 8.8 Hz)	7.15 (1H, *d*, *J* = 8.7 Hz)	7.16 (1H, *d*, *J* = 8.8 Hz)
6	6.85 (1H, *dd*, *J* = 8.3, 2.3 Hz)	6.87 (1H, *d*, *J* = 8.8 Hz)	6.82 (1H, *d*, *J* = 2.4 Hz)	6.83 (1H, *d*, *J* = 8.6 Hz)	6.85 (1H, *dd*, *J* = 8.1, 2.6 Hz)	6.87 (1H, *d*, *J* = 8.7 Hz)	6.87 (1H, *d*, *J* = 8.8 Hz)	6.86 (1H, *d*, *J* = 8.7 Hz)	6.88 (1H, *d*, *J* = 8.8 Hz)
8	6.83 (1H, *d*, *J* = 2.3 Hz)	3.99 (3H, *s*, OMe)	6.80 (1H, *d*, *J* = 2.4 Hz)	3.98 (3H, *s*, OMe)	6.82 (1H, *d*, *J* = 2.6 Hz)	3.99 (3H, *s*, OMe)	3.99 (3H, *s*, OMe)	3.99 (3H, *s*, OMe)	3.99 (3H, *s*, OMe)
1′	4.70 (2H, *d*, *J* = 6.6 Hz)	4.70 (2H, *d*, *J* = 6.7 Hz)	4.59 (2H, *d*, *J* = 6.4 Hz)	4.67 (2H, *d*, *J* = 6.4 Hz)	4.61 (2H, *d*, *J* = 6.6 Hz)	4.70 (2H, *d*, *J* = 6.5 Hz)	4.70 (2H, *d*, *J* = 6.3 Hz)	4.70 (2H, *d*, *J* = 6.8 Hz)	4.70 (2H, *d*, *J* = 6.2 Hz)
2′	5.47 (1H, *br*, *t*, *J* = 6.6 Hz)	5.49 (1H, *t*, *J* = 6.7 Hz)	5.50 (1H, *br t*, *J* = 6.4 Hz)	5.53 (1H, *br t*, *J* = 6.4 Hz)	5.53 (1H, *br t*, *J* = 6.6 Hz)	5.56 (1H, *t*, *J* = 6.5 Hz)	5.50 (1H, *br t*, *J* = 6.3 Hz)	5.54 (1H, *br t*, *J* = 6.8 Hz)	5.56 (1H, *br t*, *J* = 6.2 Hz)
4′			2.23 (1H, *dd*, *J* = 13.6, 6.0 Hz) 2.43 (1H, *dd*, *J* = 13.6, 7.7 Hz)	2.21 (1H, *dd*, *J* = 13.8, 5.8 Hz) 2.42 (1H, *dd*, *J* = 13.8, 7.8 Hz)	2.25 (2H, *m*)	2.24 (2H, *m*)	2.78 (2*H*, *d*, *J* = 4.8 Hz)	2.13 (2H, *m*)	2.16, 2.34 (each 1H, *m*)
5′	2.10 (4H, *m*)	2.10 (4H, *m*)	1.99 (3H, *s*, OAc)	1.97 (3H, *s*, OAc)	1.69 (2H, *m*)	1.70 (2H, *m*)		1.71 (2H, *m*)	1.54 (2H, *m*)
6′	5.09 (1H, *m*)	5.07 (1H, *m*)	5.68 (1H, *ddd*, *J* = 9.0, 7.7, 6.0 Hz) 5.12 (1H, *dt*, *J* = 9.0, 1.4 Hz)	5.68 (1H, *ddd*, *J* = 9.0, 7.8, 5.8 Hz) 5.11 (1H, *dt*, *J* = 9.0, 1.4 Hz)	2.71 (1H, *t*, *J* = 6.2 Hz)	2.71 (1H, *t*, *J* = 6.2 Hz)	5.63 (2H, *m*)	4.05 (1H, *m*) 1.53 (1H, *br s*, OH)	3.35 (1H, *m*) 2.32 (1H, *br d*, *J* = 4.0 Hz, OH)
7′	—	—	—	—	—	—	1.62 (1H, *s*, OH)	—	1.99 (1H, *s*, OH)
8′	1.67 (3H, *s*)	1.66 (3H, *s*)	1.71 (3H, *d*, *J* = 1.4 Hz)	1.71 (3H, *d*, *J* = 1.4 Hz)	1.30 or 1.28 (3H, *s*)	1.30 or 1.27 (3H, *s*)	1.32 (3H, *s*)	4.86 (1H, *m*) 4.94 (1H, *m*)	1.21 or 1.17 3H, *s*)
9′	1.76 (3H, *d*, *J* = 0.6 Hz)	1.75 (3H, *br*, *s*)	1.80 (3H, *d*, *J* = 0.8 Hz)	1.79 (3H, *d*, *J* = 0.4 Hz)	1.79 (3H, *d*, *J* = 0.6 Hz)	1.78 (3H, *d*, *J* = 0.8 Hz)	1.74 (3H, *d*, *J* = 0.8 Hz)	1.77 (3H, *s*)	1.77 (3H, *d*, *J* = 0.6 Hz)
10′	1.61 (3H, *s*)	1.60 (3H, *s*)	1.72 (3H, *d*, *J* = 1.4 Hz)	1.72 (3H, *d*, *J* = 1.4 Hz)	1.28 or 1.30 (3H, *s*)	1.27 or 1.30 (3H, *s*)	1.32 (3H, *s*)	1.73 (3H, *t*, *J* = 1.1 Hz)	1.17 or 1.21 (3H, *s*)

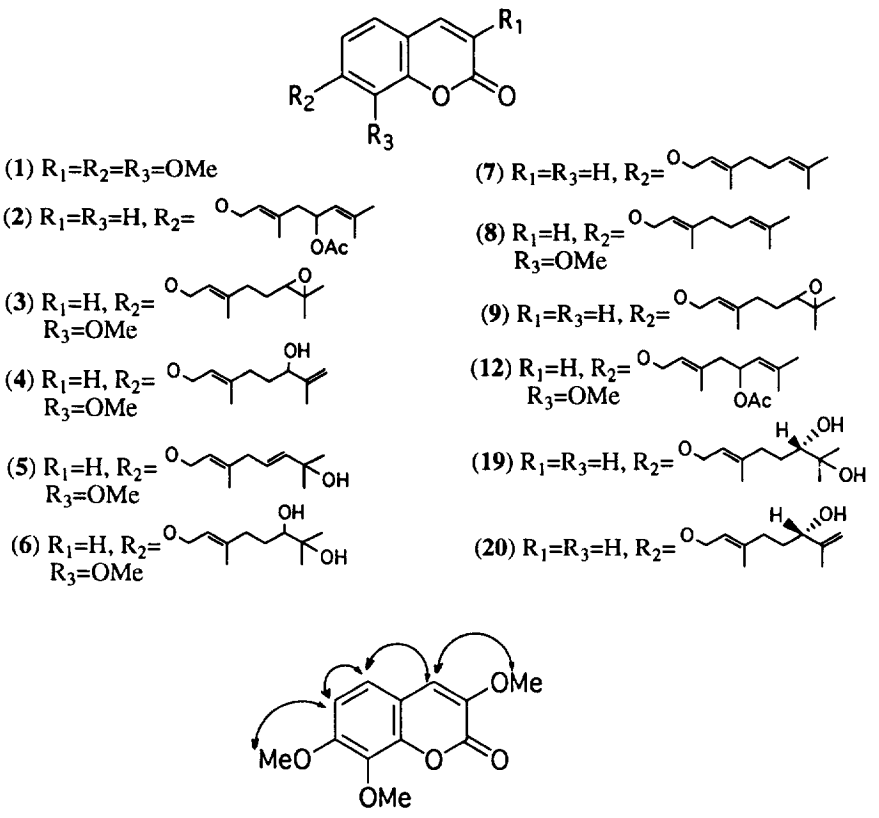

Fig. 1. NOESY correlations of **1**.

[δ5.68(1H, *ddd*, J = 9.0, 7.7, 6.0 Hz)] at C-5′ was evident in the ^{1}H NMR spectrum of **2**. This methine proton showed coupling with methylene protons [δ2.23 (1H, *dd*, J = 13.6, 6.0 Hz), δ2.43(1H, *dd*, J = 13.6, 7.7 Hz)] at C-4′ and a vinylic proton [δ5.12(1H, *dt*, J = 9.0, 1.4 Hz)] at C-6′. From the above observations and a $[M + Na]^+$ (m/z 379] in the FAB-mass spectrum, the molecular formula of **2** was found to be $C_{21}H_{24}O_5$. The presence of an acetoxy group on the ether side-chain was also supported by a significant fragment ion at m/z 297 $[M + Na - 60]^+$ in the FAB-mass spectrum. A NOESY (Fig. 2) experiment revealed that H-6′ and methyl group-8′ were in a *cis*-position and that the ether side-chain was located at C-7. HETCOR and COLOC experiments clarified the chemical shifts of four quaternary carbons on the coumarin ring at C-7 (δ163.5), C-2 (δ162.7), C-8a (δ157.3) and C-4a (δ113.5), respectively; this observation coincided with reported data [28]. From the above data, the structure of **2** was elucidated as 7-(5′-acetoxy)geranyloxycoumarin, named acetoxyaurapten; it showed laevorotatory optical activity with $[\alpha]_D^{23}$ $-30.0°$ ($CHCl_3$; c 0.05).

Epoxycollinin (**3**) was obtained as an oil. Its UV spectrum with absorptions at 258 and 320 nm also suggested the presence of a 7-oxygenated coumarin moiety. The IR spectrum showed an absorption maximum for a lactonic carbonyl group at 1725 cm^{-1}. In the ^{1}H NMR spectrum (Table 1), the chemical shifts of a methoxyl signal and

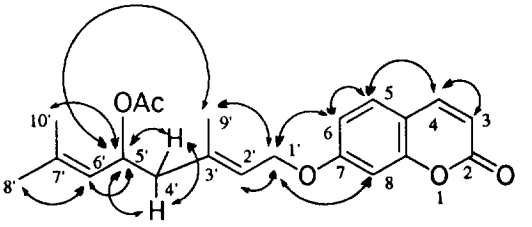

Fig. 2. NOESY correlations of **2**.

four aromatic protons in the coumarin moiety were consistent with those of collinin (**8**); a 7,8-disubstituted coumarin was suggested. Additional signals, including three methylene protons at δ4.70 (*d*, J = 6.5 Hz), 2.24 (*m*) and 1.70 (*m*), an olefinic proton at δ5.56 (*t*, J = 6.7 Hz), an epoxide methine proton at δ2.71 (*t*, J = 6.2 Hz) and three methyl protons at δ1.78 (*d*, J = 0.8 Hz), 1.27 (*s*) and 1.30 (*s*) were characteristic of a 6′,7′-epoxygeranyloxy group. Because of the small amount of **3** available, NOESY was not carried out in order to locate the position of the two substituents on the benzene ring. However, the methoxyl signal of **3** at δ3.99 is the same as those of other 7-geranyloxy-8-methoxy coumarin (**8**) or 7-oxygenated terpenyl-8-methoxy coumarins, isolated in this study; it is reasonably assigned to C-8. The structure of **3** was thus elucidated as 7-(6′,7′-epoxygeranyl)oxy-8-methoxy coumarin, which was also supported by

a $[M + Na]^+$ (m/z 367) in the FAB-mass spectrum. Compound **3** is a new compound, named epoxycollinin.

Schininallylol (**4**) was isolated as prisms. UV absorption bands at 250 sh, 256 and 332 nm also suggested the presence of a 7-oxygenated coumarin skeleton. The IR spectrum exhibited hydroxyl absorption at 3450 cm^{-1} and lactonic carbonyl absorption at 1700 cm^{-1}. The ^1H NMR spectrum (Table 1) showed the presence of a 7,8-disubstituted coumarin from the characteristic doublets of H-3 [δ6.27 (*d*, J = 9.5 Hz)], H-4[δ7.63 (*d*, J = 9.5 Hz)] and a pair of *ortho*-coupled protons of H-5 [δ7.15 (*d*, J = 8.7 Hz)] and H-6 [δ6.86 (*d*, J = 8.7 Hz)]. One methoxyl substituent was assigned to C-8 due to the methoxyl signal at δ3.99 (3H, *s*). The ^{13}C NMR spectrum indicated that there were 10 carbon atoms in the terpenyl side-chain, besides an 8-methoxycoumarin moiety. This terpenyl side-chain was elucidated as a 7-(3′,7′-dimethyl-6′-hydroxy-2′,7′-octadienyl)oxygroup from the presence of terminal methylene protons at δ4.86, 4.94 (each 1 H, *m*, H-8′), a methine proton at δ4.05 (1H, *m*, H-6′), three methylene protons at δ1.71, 2.13 (each 2H, *m*, H-5′ and H-4′), 4.70 (2H, *d*, J = 6.8 Hz, H-1′), one olefinic proton at δ5.54 (*br t*, J = 6.8 Hz, H-2′), two vinylic methyl protons at δ1.73 (3H, *t*, J = 1.1 Hz, 10′-Me) and 1.77(3H, *s*, 9′-Me) and one hydroxyl group at δ1.53 (*br s*, 6′-OH). Coupling of H-5′ with the vicinal H-4′ and H-6′ was observed in a COSY experiment and a M_r of 344 was determined from the FAB-mass spectrum ($[M + Na]^+$, m/z 367); both of these observations further supported the above structure of a terpenyl ether chain. Finally, a NOE difference experiment (Fig. 3) showed that H-1′ had correlation with H-6 and 8-OMe in the coumarin moiety and also with H-2′ and 9′-Me. All this evidence supported the structure of **4** as 7-(3′,7′-dimethyl-6′-hydroxy-2′,7′-octadienyl)oxy-8-methoxy coumarin, a new compound with $[\alpha]_D^{22}$ − 16.4° ($CHCl_3$; *c* 0.07) named schininallylol.

Schinilenol (**5**) was obtained as an oil with a M_r of 344 determined by FAB-mass spectrometry ($[M + Na]^+$, m/z 367). UV absorptions at 250 sh, 256 and 319 nm again showed a 7-oxygenated coumarin moiety in the molecule. The IR spectrum exhibited a hydroxyl group at 3450 cm^{-1} and lactonic carbonyl group at 1720 cm^{-1}. In the ^1H NMR spectrum (Table 1), the splitting patterns of two pairs of doublets [δ6.27 and 7.63 (each 1H, *d*, J = 9.5 Hz), δ7.16 and 6.87 (each 1H, *d*, J = 8.8 Hz)] and one methoxyl signal at δ3.99 (3H, *s*) were consistent with those of collinin (**8**); a 7,8-disubstituted coumarin was thus suggested. An oxygenated terpenyl ether chain, located at C-7, was elucidated as 3′,7′-dimethyl-7′-hydroxy-2′,5′-octadienyl)oxy due to the existence of three vinylic protons at δ5.50 (1H, *br t*, J = 6.3 Hz, H-2′), 5.63 (2H, *m*, H-5′ and H-6′), vinylic methyl protons at δ1.74 (3H, *d*, J = 0.8 Hz, 9′-Me), two methylene protons at δ2.78 (2H, *d*, J = 4.8 Hz, H-4′), 4.70 (2H, *d*, J = 6.3 Hz), two methyl protons at δ1.32 (6H, *s*, 8′-Me and 10′-Me) and a tertiary hydroxyl group at δ1.62 (1H, *s*, disappeared after addition of D_2O, 7′-OH). According to these observations, **5** was elucidated as 7-(3′,7′-dimethyl-7′-hydroxy-2′,5′-octadienyl)oxy-8-methoxy coumarin. Its structure was further confirmed by HETCOR. ^3C NMR, DEPT and NOESY (Fig. 4) experiments.

Schinindiol (**6**) was isolated as prisms with an M_r of 362 from the FAB-mass spectrum ($[M + Na]^+$, m/z 385). UV absorption bands at 248sh, 256 and 320 nm demonstrated the presence of a 7-oxygenated coumarin moiety. The IR spectrum exhibited a hydroxyl absorption at 3450 cm^{-1} and a lactonic carbonyl absorption at 1720 cm^{-1}. In the ^1H NMR spectrum (Table 1), there were two sets of AB doublets, δ6.27 and 7.64 (each 1H, *d*, J = 9.5 Hz), δ6.88 and 7.16 (each 1H, *d*, J = 8.8 Hz), corresponding to H-3 and H-4, and H-6 and H5, and a methoxyl singlet at δ3.99, which was characteristic of a 7-substituted 8-methoxy coumarin. Further analyses of the ^{13}C NMR spectrum showed the presence of 20 carbons in **6**; a methoxy signal at δ62.0 and the presence of a terpenyl ether chain located at C-7 of a coumarin moiety was also suggested. The structure of the hydroxylated terpenyl ether chain was proposed as 7-(6′,7′-dihydroxy-3′,7′-dimethyl-2′-octenyl)oxy due to a vinylic proton at δ5.56 (*br t*, J = 6.2 Hz, H-2′), vinylic methyl protons at δ1.77 (*d*, J = 0.6 Hz, 9′-Me), three methylene protons at δ2.16, 2.34 (each 1H, *m*, H-4′), 1.54 (2H, *m*, H-5′) and 4.70 (2H, *d*, J = 6.2 Hz, H-1′), a methine proton at δ3.35 (1H, *m*, H-6′), two methyl protons at δ1.17 and 1.21 (each 3H, *s*), and two hydroxyl protons at δ2.32 (*br d*, J = 4.0 Hz, 6′-OH) and 1.99 (*s*, 7′-OH); these signals were identical to those of the 7-oxygenated side-chain of marmin (**19**) [29]. From the above data, schinindiol was identified as 7-(6′,7′-dihydroxy-3′,7′-dimethyl-2′-octenyl)oxy-8-methoxy coumarin, which was further confirmed by NOE difference (Fig. 5), COSY, DEPT and HETCOR experiments. This new compound was named

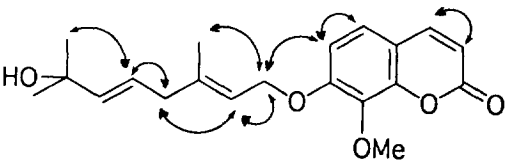

Fig. 4. NOESY correlations of **5**.

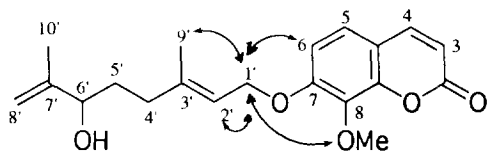

Fig. 3. NOE correlations of **4**.

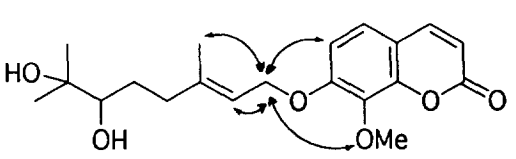

Fig. 5. NOE correlations of **6**.

as schinindiol (**6**) and it showed laevorotatory optical activity with $[\alpha]_D^{22}$ − 22.0° ($CHCl_3$; *c* 0.05).

Recently, schinifolin and acetoxyschinifolin were reported as new compounds by Hong *et al.* [15]. However, the former is the same as collinin (**8**) [21]. Therefore, the latter should be renamed as acetoxycollinin, which showed dextrorotatory optical activity (−)-Acetoxycollinin (**12**) with $[\alpha]_D^{23}$ − 32.8° ($CHCl_3$, *c* 0.05) has now been isolated from nature for the first time in the present study.

Without an enantiomeric antipode for comparison, but with reference to the *R*-configuration of (+)-marmin (**19**) [29] and (+)-allylic alcohol (**20**) [29], the stereochemistry at C-6′ of both (−)-schinindiol (**6**) and (−)-schininallylol (**4**) would appear to be of the *S*-configuration.

The $CHCl_3$-soluble fraction of the bark of this species showed strong anti-platelet activity *in vitro* using the turbidimetric method [30]. Bioassay-guided fractionation led to the isolation of six coumarins, schinicoumarin (**1**), acetoxyaurapten (**2**), schininallylol (**4**), aurapten (**7**), collinin (**8**), (−)-acetoxycollinin (**12**) and one alkaloid, dictamnine (**15**) [31] as the active principles with anti-platelet aggregation activity (Table 2). Among the isolates, aurapten (**7**), and collinin (**8**) are the major components, which, even at 50 μg ml^{-1}, caused complete inhibition of platelet aggregation induced by arachidonic acid and collagen *in vitro*. The presence of 7-terpenyl or oxygenated 7-terpenyl groups enhanced the inhibitory activity on platelet aggregation *in vitro* compared with 7-hydroxy coumarin (**11**) or 7-hydroxy-8-methoxy coumarin (**10**) and was well evidenced from our study.

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR: $CDCl_3$ with TMS as int. standard. MS: 70 eV. CC: silica gel 60 (70–230 mesh) (Merck). TLC and prep. TLC: silica gel 60 GF 254 (Merck).

Plant material. Zanthoxylum schinifolium Sieb. & Zucc. was collected at Wutai, Pingtung Hsien, Taiwan, in August 1989. A voucher specimen is deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Taiwan, Republic of China.

Extraction and separation. Dried root and stem bark (3.4 kg) was extracted with warm MeOH. There was a large ppt. (34.7 g) which was removed by filtration when the MeOH soln was concd *in vacuo*. The concd filtrate was partitioned with $CHCl_3$–H_2O (1 : 1) to afford a $CHCl_3$-soluble Fr. (185.6 g). A portion of this fr. (26.8 g) was chromatographed on a silica gel column and eluted with $CHCl_3$ enriched with MeOH to obtain A_1(7.2 g, $CHCl_3$), A_2(3.2 g, $CHCl_3$) and A_3 (9.5 g, $CHCl_3$–MeOH, 50 : 1) frs. Fr. A_1 was washed with Et_2O to afford crude crystals (495 mg). A part of this fr. (80 mg) was purified by prep. TLC (*n*-hexane–EtOAc, 5:1) and recrystallization to obtain **7** (40 mg). The Et_2O washings (6.7 g) were chromatographed on a silica gel column with *n*-hexane enriched with EtOAc to obtain 5 frs : fr. A_1-a (1.2 g, *n*-hexane–EtOAc, 70 : 1), fr. A_1-b (79.1 mg, *n*-hexane–EtOAc, 50 : 1), fr. A_1-c (1.5 g, *n*-hexane–EtOAc, 30 : 1), fr. A_1-d (1.4 g, *n*-hexane–EtOAc, 20 : 1) and fr. A_1-e (902 mg, *n*-hexane–EtOAc, 10 : 1). Fr. A_1-a was washed with MeOH to yield **17** (44.4 mg) after recrystallization from $CHCl_3$–MeOH. Fr. A_1-b was purified by prep. TLC

Table 2. Inhibitory effect of compounds **1**, **2**, **4**, **7**, **8**, **10**, **11** and **12** on the aggregation of washed rabbit platelets*

Treatment	Concentration (μg ml^{-1})	% Aggregation AA	Collagen	PAF
Control		86.4 ± 1.0	89.7 ± 1.1	90.5 ± 1.0
1	100	0.0 ± 0.0^c	76.8 ± 4.1	89.1 ± 1.2
2	100	0.0 ± 0.0^c	0.0 ± 0.0^c	9.7 ± 7.9^c
4	100	0.0 ± 0.0^c	23.9 ± 5.2^c	54.9 ± 10.4^c
7	100	0.0 ± 0.0^c	2.9 ± 2.6^c	0.0 ± 0.0^c
	50	2.2 ± 1.9^c	19.8 ± 17.1^c	—
	20	86.7 ± 1.5	87.1 ± 0.4	—
8	100	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c
	50	0.0 ± 0.0^c	0.0 ± 0.0^c	—
	20	40.9 ± 20.5^a	83.4 ± 1.0^c	—
10	100	79.1 ± 2.0^b	81.0 ± 0.8^c	91.0 ± 1.6
11	100	76.6 ± 2.1^c	76.3 ± 2.7^c	87.1 ± 2.6
12	100	0.0 ± 0.0^c	5.4 ± 4.4^c	0.0 ± 0.0^c

*Platelets were pre-incubated with each compound or 0.5% DMSO (control) at 37° for 3 min, then the inducer arachidonic acid (AA, 100 μM), collagen (10 μg ml^{-1}) or PAF (2 ng ml^{-1}) was added to trigger aggregation. Percentages of aggregation are presented as measurement ± SEM (n = 3–5).

$^aP < 0.1$; $^bP < 0.01$; $^cP < 0.001$ as compared with the respective control.

(benzene), then recrystallized from MeOH to give **18** (6.6 mg). Washing with Et_2O and purification of fr. A_1-c produced **7** (259 mg) again. A part (102 mg) of fr. A_1-d was purified by prep. TLC (benzene–EtOAc, 10:1) to yield **8** (94.5 mg) and **9** (1.1 mg). Fr. A_1-e was chromatographed, over silica gel to yield an eluate (13.9 mg, *n*-hexane–EtOAc, 12:1), which was further purified by prep. TLC (*n*-hexane–EtOAc 1:1) to afford **2** (11 mg). Fr. A_2 was chromatographed on a silica gel column with benzene enriched with EtOAc to give 3 frs: fr. A_2-a (1.0 g, benzene–EtOAc, 20:1), fr. A_2-b (167 mg, benzene–EtOAc, 10:1) and fr. A_2-c (130 mg, benzene–EtOAc, 1:1). Fr. A_2-a was washed with MeOH, then purified by recrystallization to obtain **14**. The MeOH washings were chromatographed on a silica gel column to provide fr. A_2-a-1 (46 mg, *n*-hexane–EtOAc, 10:1) and fr. A_2-a-2 (40.2 mg, *n*-hexane–EtOAc 5:1). The former fr. was purified by prep. TLC (*n*-hexane–EtOAc 2:1) to obtain **15** (12 mg) and **3** (2.0 mg). The latter fr. was also purified by prep. TLC ($CHCl_3$–EtOAc, 4:1) to afford **10** (13.6 mg) and **11** (6.3 mg). Fr. A_2-b was subjected to silica gel CC to yield fr. A_2-b-1 (63 mg, benzene–EtOAc, 5:1) and fr. A_2-b-2 (50 mg, benzene–EtOAc, 1:1). Purification by prep. TLC produced **5** (46.4 mg) with benzene–EtOAc (1:1) from fr. A_2-b-1 and **4** (19.8 mg) with *n*-hexane EtOAc (2:1) from fr. A_2-b-2. A part (50 mg) of fr. A_2-c was purified by prep. TLC (benzene–EtOAc (2:1) to obtain **6** (20 mg). Fr. A_3 was washed with Et_2O, then purified by recrystallization to give **1** (31.9 mg). The Et_2O washings were purified by recrystallization to yield **16** (200 mg). The mother liquor of **1** was subjected to silica gel CC to afford fr. A_3-a (92 mg) C_6H_6) and fr. A_3-b (benzene–EtOAc, 10:1). Fr. A_3-a was purified by prep. TLC (benzene–EtOAc, 4:1) to give **12** (46 mg) and fr. A_3-b was rechromatographed on a silica gel column and from the *n*-hexane–EtOAc (4:1) eluate afforded **13** (1.4 mg) after purification by recrystallization.

Schinicoumarin (**1**). Needles, mp 147–151°. IR ν_{max}^{KBr} cm^{-1}: 1730 (C=O). UV λ_{max}^{EtOH} nm (log ε): 240 (3.86), 260 sh (3.75), 318 (4.19). ^1H NMR (200 MHz): δ3.89 (3H, *s*, C-3-OMe), 3.93 (3H, *s*, C-7-OMe), 3.99 (3H, *s*, C-8-OMe), 6.78 (1H, *s*, H-4), 6.87 (1H, *d*, *J* = 8.8 Hz, H-6), 7.18 (1H, *d*, *J* = 8.8 Hz, H-5). ^{13}C NMR (50 MHz): δ56.2 (C-3-OMe) 56.4 (C-7-OMe), 61.5 (C-8-OMe), 109.2 (C-6), 113.1 (C-4a), 114.2 (C-3), 120.8 (C-5), 136.3 (C-8), 142.7 (C-4), 143.6 (C-8a), 153.1 (C-7), 157.4 (C-2). EIMS *m/z* (rel int.): 236 $[M]^+$ (100); HRMS: found $[M]^+$ 236.0695: $C_{12}H_{12}O_5$, requires 236.0685.

Acetoxyaurapten (**2**). Prisms, mp 53–55°. IR ν_{max}^{KBr} cm^{-1}: 1740, 1722 (C=O). UV λ_{max}^{EtOH} nm (log ε): 250 sh (2.87), 324 (3.68). ^1H NMR: Table 1. ^{13}C NMR (50 MHz): δ17.3 (C-10′), 18.6 (C-9′), 21.4 (OCOMe), 25.9 (C-8′), 45.5 (C-4′), 65.8 (C-1′), 70.0 (C-5′), 102.5 (C-8), 113.5 (C-4a), 114.1 (C-6 or C-3), 114.1 (C-3 or C-6), 123.0 (C-2′), 124.4 (C-6′), 129.9 (C-5), 138.8 (C-7′), 139.0 (C-3′), 144.7 (C-4), 157.3 (C-8a), 162.7 (C-2), 163.5 (C-7), 171.8 (OCOMe). FAB-MS *m/z* (rel. int.): 379.15 $[M + Na]^+$ (36). $[\alpha]_D^{23}$: −30.0° ($CHCl_3$; *c* 0.05).

Epoxycollinin (**3**). Oil IR ν_{max}^{neat} cm^{-1}: 1725 (C=O). UV λ_{max}^{EtOH} nm (log ε): 258 sh (3.87), 320 (4.21). ^1H NMR: Table 1. FAB-MS *m/z* (rel. int.): 367.07 $[M + Na]^+$ (100).

Schininallylol (**4**). Prisms, mp 78–80°. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1700 (C=O). UV λ_{max}^{EtOH} nm (log ε): 250 sh (3.65), 256 (3.67), 332 (4.04). ^1H NMR: Table 1. ^{13}C NMR (50 MHz): δ17.0 (C-9′ or C-10′), 17.7 (C-10′ or C-9′), 33.0 (C-5′), 35.8 (C-4′), 62.0 (OMe), 66.9 (C-1′), 76.1 (C-6′), 111.3 (C-6), 112.3 (C-9′), 114.5 (C-3), 114.8 (C-4a), 120.3 (C-2′), 123.7 (C.5), 138.0 (C-8), 142.9 (C-3′), 144.9 (C-4), 148.6 (C-7′), 149.5 (C-8a), 156.3 (C-8), 162.1 (C-2). FAB-MS *m/z* (rel. int.): 367.15 $[M + Na]^+$ (16.9). $[\alpha]_D^{22}$: −16.4° ($CHCl_3$; *c* 0.07).

Schinilenol (**5**). Oil. IR ν_{max}^{neat} cm^{-1}: 3450 (OH), 1720 (C=O). UV λ_{max}^{EtOH} nm (log ε): 250 sh (3.57), 256 (3.59), 319 (4.07). ^1H NMR: Table 1. ^{13}C NMR (50 MHz): δ16.9 (C-9′) 30.1 (C-8′, C-10′), 42.5 (C-4′), 62.0 (OMe), 66.9 (C-1′), 71.3 (C-7′), 111.3 (C-6), 114.5 (C-3), 114.8 (C-4a), 120.9 (C-2′), 123.7 (C-5), 125.0 (C-6′), 138.0 (C-8), 141.6 (C-5′), 141.8 (C-3′), 144.9 (C-4), 149.5 (C-8a), 156.3 (C-7), 162.0 (C-2). FAB-MS *m/z* (rel. int.): 367.45 $[M + Na]^+$ (81).

Schinindiol (**6**). Prisms, mp 63–65°. IR ν_{max}^{neat} cm^{-1}: 3450 (OH), 1720 (C=O). UV λ_{max}^{EtOH} nm (log ε): 248 sh (3.34), 256 (3.38), 320 (3.90). ^1H NMR: Table 1. ^{13}C NMR (50 MHz): δ16.9 (C-10′), 23.5 (C-8′), 26.8 (C-9′), 29.7 (C-5′), 36.8 (C-4′), 62.0 (OMe), 66.9 (C-1′), 73.7 (C-7′), 78.6 (C-6′), 111.3 (C-6), 114.5 (C-3), 114.8 (C-4a), 120.4 (C-2′), 123.7 (C-5), 138.0 (C-8), 143.1 (C-3′), 144.9 (C-4), 149.5) (C-8a), 159.3 (C-7), 162.1. (C-2). FAB-MS *m/z* (rel. int.): 385.29 $[M + Na]^+$ (100). $[\alpha]_D^{22}$: −22.0° ($CHCl_3$; *c* 0.05).

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