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COUMARINS AND ANTI-HBV CONSTITUENTS FROM *ZANTHOXYLUM SCHINIFOLIUM*

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Key Word Index—*Zanthoxylum schinifolium*; Rutaceae; bark; coumarins; terpenyl coumarin; lignan; alkaloid; 7-(5′,6′-dihydroxy-3′,7′-dimethylocta-2′,7′-dienyloxy)-coumarin; 7-(2′,6′-dihydroxy-7′-methyl-3′-methyleneocta-7′-enyloxy)-8-methoxycoumarin; anti-HBV DNA replication.

Abstract—Continuing examination on the chloroform-soluble part of the bark of *Zanthoxylum schinifolium*, two new terpenyl coumarins, 7-(5′,6′-dihydroxy-3′,7′-dimethylocta-2′,7′-dienyloxy)-coumarin and 7-(2′,6′-dihydroxy-7′-methyl-3′-methyleneocta-7′-enyloxy)-8-methoxycoumarin, along with three coumarins, anisocoumarin H, 7-[(*E*)-7′-hydroxy-3′,7′-dimethylocta-2′,5′-dienyloxy]-coumarin, scopoletin; two alkaloids, 4-methoxy-l-methyl-2-quinolone and oxynitidine and a lignan, (+)-matairesinol, were isolated as additional constituents. The structures of these compounds were elucidated by spectral analyses. Among the isolates of the bark, collinin and oxynitidine showed significant activity of anti-HBV DNA replication.

INTRODUCTION

Zanthoxylum schinifolium Sieb. & Zucc. is distributed in China, Korea, Japan and Taiwan [1]. In China, the ripe pericarp of the fruit is one of the sources of Pericarpium Zanthoxyli [2]. The methanol extract of the bark of Formosan species shows strong anti-platelet aggregation activity. Consequently, new terpenyl coumarins, along with several anti-platelet aggregation constituents, were isolated from the chloroform-soluble part [3]. Continuing examination of the same part of this plant, two new terpenyl coumarins, 7-(5′,6′-dihydroxy-3′,7′-dimethylocta-2′,7′-dienyloxy)-coumarin (**1**) and 7-(2′,6′-dihydroxy-7′-methyl-3′-methyleneocta-7′-enloxy)-8-methoxy-coumarin (**2**), three coumarins, anisocoumarin H (**3**) [4], 7-[(*E*)-7′-hydroxy-3′,7′-dimethylocta-2′,5′-dienyloxy]-coumarin (**4**) [5] and scopoletin (**5**) [6], two alkaloids, 4-methoxy-l-methyl-2-quinolone (**6**) [7] and oxynitidine (**7**) [8], one lignan, (+)-matairesinol (**8**) [9], have been isolated. All of these known compounds were identified by comparisons of their IR, UV, ^{1}H NMR, TLC and/or mmp with corresponding authentic samples or literature data. In another screening program of anti-HBV DNA replication activity on Formosan plants, the methanol extract of the bark of this plant showed the ability to inhibit hepatitis B virus (HBV) DNA replication in a HBV-transfected cell line (2.2.15). This paper reports, the structure elucidation of these new compounds and the constituents with anti-HBV DNA replication activity.

RESULTS AND DISCUSSION

7-(5′,6′-Dihydroxy-3′,7′-dimethylocta-2′,7′-dienyloxy)-coumarin (**1**) was isolated as a colourless oil. Its molecular formula of $C_{19}H_{22}O_5$ was established by FAB-mass spectrometry ($[M+H]^+$, m/z 331). The UV spectrum, with absorptions at 260 and 324 nm, suggested the presence of a 7-oxygenated coumarin moiety. The IR spectrum exhibited a lactonic carbonyl absorption at 1700 cm^{-1} and a hydroxyl absorption at 3400 cm^{-1}. The ^{1}H NMR spectrum also showed the presence of a 7-oxygenated coumarin from the characteristic doublets of H-3 (δ 6.25, *d*, J = 9.4 Hz) and H-4 (δ 7.63, *d*, J = 9.4 Hz), a pair of *ortho*-coupled protons of H-5 (δ 7.36, *d*, J = 8.4 Hz), and H-6 (δ 6.85, *dd*, J = 8.4, 2.2 Hz) which was *meta*-coupled with H-8 (δ 6.82, *d*, J = 2.2 Hz). The signals of a terpenyloxyl substituent at C-7 including H-1′, H-2′, H-4′, H-8′, 9′-CH_3 and 10′-CH_3 were similar with those of schininallylol (**9**) [3] and suggested an octadienyloxyl skeleton. The residual two methine protons at δ 3.90 (*d*, J = 5.6 Hz) and 3.82 (*m*), were

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Table 1. ^{1}H NMR data for compounds **1**, **9** and **2**

H	1	9*	2*
3	6.25 (1H, *d*, *J* = 9.4 Hz)	6.27 (1H, *d*, *J* = 9.5 Hz)	6.29 (1H, *d*, *J* = 9.5 Hz)
4	7.63 (1H, *d*, *J* = 9.4 Hz)	7.63 (1H, *d*, *J* = 9.5 Hz)	7.63 (1H, *d*, *J* = 9.5 Hz)
5	7.36 (1H, *d*, *J* = 8.4 Hz)	7.15 (1H, *d*, *J* = 8.7 Hz)	7.16 (1H, *d*, *J* = 8.7 Hz)
6	6.85 (1H, *dd*, *J* = 8.4, 2.2 Hz)	6.86 (1H, *d*, *J* = 8.7 Hz)	6.89 (1H, *d*, *J* = 8.7 Hz)
8	6.82 (1H, *d*, *J* = 2.2 Hz)	3.99 (3H, *s*, OMe)	4.00 (3H, *s*, OMe)
1′	4.63 (2H, *d*, *J* = 6.4 Hz)	4.70 (2H, *d*, *J* = 6.8 Hz)	4.07, 4.18 (each 1H, *m*)
2′	5.61 (1H, *br t*, *J* = 6.4 Hz)	5.54 (1H, *br t*, *J* = 6.8 Hz)	4.56 (1H, *m*)
4′	2.26 (2H, *m*)	2.13 (2H, *m*)	2.22 (2H, *m*)
5′	3.82 (1H, *m*)	1.71 (2H, *m*)	1.81 (2H, *m*)
6′	3.90 (1H, *d*, *J* = 5.6 Hz)	4.05 (1H, *m*)	4.12 (1H, *m*)
8′	5.00, 5.05 (each 1H, *m*)	4.86, 4.94 (each 1H, *m*)	4.86, 4.97 (each 1H, *br s*)
9′	1.83 (3H, *s*)	1.77 (3H, *s*)	5.07, 5.26 (each 1H, *br s*)
10′	1.77 (3H, *t*, *J* = 1.0 Hz)	1.73 (3H, *t*, *J* = 1.1 Hz)	1.74 (3H, *br s*)
OH	2.08 (1H, *br s*) one not observed.	1.53 (1H, *br s*, OH-6′)	2.95 (1H, *br s*) one not observed

* Measured at 200 MHz.

assigned to C-6′ and C-5′ which connected each to a hydroxyl group, respectively, according to signal complexity and chemical shift. From the above data, the structure of **1** was elucidated as 7-(5′,6′-dihydroxy-3′,7′-dimethylocta-2′,7′-dienyloxy)-coumarin.

7-(2′,6′-Dihydroxy-7′-methyl-3′-methyleneocta-7′-enyloxy)-8-methoxycoumarin (**2**) was isolated as colourless oil. Its molecular formula of $C_{20}H_{24}O_6$ was determined by FAB-mass spectrometry ($[M+H]^+$, *m/z* 361). The IR spectrum indicated the presence of a hydroxyl group at 3450 cm^{-1} and a lactonic carbonyl group at 1725 cm^{-1}. UV absorptions at 267, 320 nm showed the existence of a 7-oxygenated coumarin. In the ^{1}H NMR spectrum (Table 1), there were two sets of AB doublets, δ 6.29 and 7.63 (each 1H, *d*, *J* = 9.5 Hz), δ 6.89 and 7.16 (each 1H, *d*, *J* = 8.7 Hz) corresponding to H-3 and H-4, H-6 and H-5, and a methoxyl singlet at δ 4.00, which was characteristic of a 7-substituted 8-methoxycoumarin. The proton signals (H-4′ ~ H-8′, H-10′) on the last six carbons in the terpenyloxyl substituent were similar with those of **9** with a terminal methylene at C-7′. Two olefinic protons on second terminal methylene at δ 5.07, 5.26 (each *br s*) were attributed to H-9′. A methine proton at δ 4.56 (*m*), two nonequivalent *O*-benzylic protons (δ 4.07, 4.18, each 1H, *m*) were assigned to C-2′ and C-1′, respectively, and a hydroxyl group was attached to C-2′. From the above data, the structure of **2** was elucidated as 7-(2′,6′-dihydroxy-7′-methyl-3′-methyleneocta-7′-enyloxy)-8-methoxycoumarin which was further supported by ^{1}H–^{1}H COSY and NOESY experiment (Fig. 1). Owing to the small amount of **1** and **2**, the measurement of the specific rotation was not carried out.

The chloroform-soluble fraction of the bark of this species showed activity of anti-HBV DNA replication *in vitro* using the method as described in the literature [10]. Among the isolates from the chloroform-soluble fraction of the bark, collinin (**10**) [3] and oxynitidine

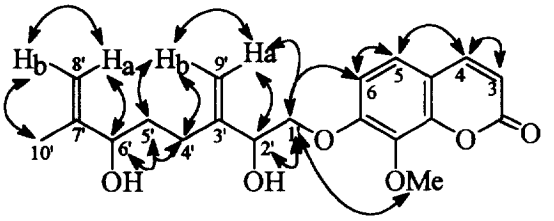


Fig. 1. NOESY correlations of **2**.

(**7**) exhibited anti-HBV activity and showed ID_{50} value of 17.1 μg ml^{-1} and 30.8 μg ml^{-1}, respectively (Table 2). A cytotoxicity test was performed and the ED_{50} and $HBID_{50}$ values were shown in Table 2. Their selective index values were equivalent to ddC though not as potent as sddC [10].

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR: $CDCl_3$ with TMS as int. standard. MS: 70 eV. CC: silica gel (70–230 mesh, 230–400 mesh) (Merck). TLC and prep. TLC: silica gel 60 F 254 (Merck).

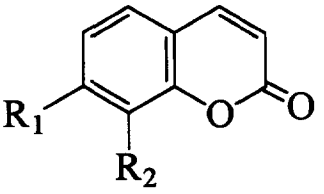
Table 2. Comparative potencies of **7** and **10** as monitored by anti-HBV and cytotoxicity

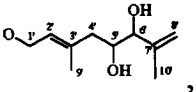
Compound	ED_{50} (μg ml^{-1})	$HBID_{50}$ (μg ml^{-1})	SI
7	>200	30.8	>6.49
10	68.3	17.1	3.99

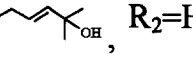
ED_{50}: concentration that caused a 50% reduction in cell number.

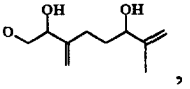
$HBID_{50}$: concentration that inhibited HBV viral DNA yield in the medium by 50%.

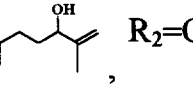
SI: selective index ($ED_{50}/HBID_{50}$).

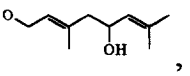


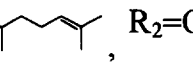
(1) R_1=  R_2=H

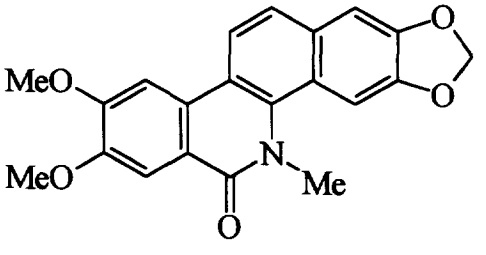
(4) R_1=  R_2=H

(2) R_1=  R_2=OMe

(9) R_1=  R_2=OMe

(3) R_1=  R_2=H

(10) R_1=  R_2=OMe



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Plant material. Zanthoxylum schinifolium 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. The extraction procedure was described in our previous paper [3]. The mother liquor of fr. A_3 was chromatographed over silica gel eluting with benzene and gradually increasing the polarity with EtOAc and 13 frs. (A_3-a ~ A_3-m) were collected. The isolation of fr. A_3-a and fr. A_3-b was reported [3]. The fr. A_3-c (446 mg, benzene–EtOAc, 10:1) was rechromatographed on a silica gel column to obtain fr. A_3-c-1 (112 mg, *n*-hexane–EtOAc, 10:3), fr. A_3-c-2 (32 mg, *n*-hexane–EtOAc, 10:3), fr. A_3-c-3 (65.6 mg, *n*-hexane–EtOAc, 10:3), fr. A_3-c-4 (53.1 mg, *n*-hexane–EtOAc, 1:1), fr. A_3-c-5 (36.8 mg, *n*-hexane–EtOAc, 1:1), fr. A_3-c-6 (14.3 mg, *n*-hexane–EtOAc, 1:1) and fr. A_3-c-7 (16.9 mg, *n*-hexane–EtOAc, 1:1). Fr. A_3-c-2 was purified by prep. TLC ($CHCl_3$–$MeCO_2$–MeOH, 50:3:1) to obtain umbelliferone (1.1 mg), skimmianine (1.6 mg), **3** (5.3 mg) and **4** (5.1 mg). Fr. A_3-c-4 was purified by chromatography to obtain fr. A_3-c-4-1 (16.4 mg, $CHCl_3$–Me_2CO, 20:1) and fr. A_3-c-4-2 (28.7 mg, $CHCl_3$–Me_2CO, 20:1). The former fr. was purified by prep. TLC ($CHCl_3$–MeOH, 30:1) to give **3** (2.8 mg) again. The latter fr. was treated in the same way as the former fr. to yield **8** (23.6 mg). The fr. A_3-c-5 was purified by prep. TLC ($CHCl_3$–MeOH, 30:1) to give **8** (6.4 mg) and **7** (3.1 mg). Fr. A_3-c-6 was purified by prep. TLC (*n*-hexane–$CHCl_3$–EtOAc, 1:5:1) to obtain **7** (3.1 mg). Compound **1** (1.7 mg) was obtained from fr. A_3-c-7 purified using prep. TLC ($CHCl_3$–MeOH, 20:1). The fr. A_3-d (116.7 mg, benzene–EtOAc, 10:1) was chromatographed on silica gel to obtain fr. A_3-d-1 (32.1 mg, *n*-hexane–EtOAc, 10:3), fr. A_3-d-2 (18 mg, *n*-hexane–EtOAc, 1:1), fr. A_3-d-3 (5.7 mg, *n*-hexane–EtOAc, 3:7) and fr. A_3-d-4 (23.5 mg, EtOAc). Fr. A_3-d-2 was purified by prep. TLC (CH_2Cl_2–EtOAc, 5:2) to yield **2** (2.4 mg) and **6** (1.7 mg). Fr. A_3-d-3 was purified by prep. TLC (benzene–CH_2Cl_2, 1:3) to obtain **5** (2.1 mg).

7-(5′,6′-Dihydroxy-3′,7′-dimethylocta-2′,7′-dienyloxy)-coumarin (**1**). Colourless oil. FAB-MS *m/z*: 331 $[M+H]^+$. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 1700 (C=O). UV λ_{max}^{EtOH} nm (log ε): 324 (3.41), 260 (2.52). ^1H NMR (400 MHz): see Table 1.

7-(2′,6′-Dihydroxy-7′-methyl-3′-methyleneocta-7′-enyloxy)-8-methoxycoumarin (**2**). Colourless oil. FAB-

MS *m/z*: 361 $[M+H]^+$, IR ν_{max}^{neat} cm^{-1}: 3450 (OH), 1725 (C=O), UV λ_{max}^{EtOH} nm (log ε): 320 (3.51), 267 (2.81). ^1H NMR (200 MHz): see Table 1.

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