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TWO COUMARINS FROM THE ROOT BARK OF *CLAUSENA EXCAVATA*

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Key Word Index—*Clausena excavata*; Rutaceae; coumarin; flavonoid; claucavatin-A; claucavatin-B.

Abstract—Two new pyrano-coumarins, claucavatin-A and -B, together with eight known coumarins and two known flavonoids were isolated and identified from the acetone extract of the root bark of *Clausena excavata*. Their structures were elucidated by the spectroscopic analyses. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Clausena excavata has been used as a folk medicine in the treatment of snake-bite and as a detoxification agent [1]. We have reported the isolation of a strong antibacterial compound, nordentatin (**6**), along with some coumarins and carbazole alkaloids from the root bark of *C. excavata* [2]. After reinvestigating the acetone extract, two new coumarins, claucavatin-A (**1**) and -B (**2**), together with eight known coumarins, kinocoumarin (**3**) [3], clausenidin (**4**) [2], clausarin (**5**) [2, 4], nordentatin (**6**) [2, 4], xanthoxyletin (**7**) [2, 4], xanthyletin (**8**) [2, 4], osthol (**9**) [5] and cedrelopsin (**10**) [6], two known flavonoids, isoliquiritigenin (**11**) [7, 8] and liquiritigenin (**12**) [8], and several carbazole alkaloids were obtained. Here we report the structural elucidation of the two new coumarins by spectroscopic analyses.

RESULTS AND DISCUSSION

Comparison of the UV, IR and ^{1}H NMR spectra of **1** and **2** with the previously recorded spectra **3**, **4**, **5** and **6** suggested that a 5,7-dioxycoumarin ring system is involved in the structures of **1** and **2** [9, 10]. A dimethylpyranyl ring fused to a coumarin skeleton was also found and the junction of these two units was found to be between C-6 and C-7, rather than C-5 and C-6.

Claucavatin-A (**1**) has the molecular formula $C_{24}H_{28}O_5$ as determined by high resolution mass spectrometry. The ^{1}H NMR spectrum of **1** showed similar absorptions to those of **4** or **5** (Table 1). The broad IR band at 3450 cm^{-1} and the low-field ^{1}H NMR peak at δ 13.00 indicated a strongly intramolecular hydrogen bonded phenolic proton (5-OH) located at the *peri* position of a carbonyl group. A methylene singlet at δ 2.73 and a *gem*-dimethyl singlet at δ 1.62 indicated a 2′-2′-dimethyl-4-pyranone ring linearly attached to the coumarin ring. Unlike the two *cis*-vinyl protons found in **4**, in **1** only one downfield singlet proton was seen (δ 7.89). This suggests that there is a substituent attached to C-3. The remaining peaks disclosed pairs of 1,1-dimethylallyl groups at δ 1.46 and 1.47 (each 6H, *s*, each $2 \times CH_3$), 4.88 and 5.09 (each 1H, *dd*, each J = 10.4, 0.8 Hz), 4.92 and 5.10 (each 1H, *dd*, J = 17.8, 0.8, and 17.0, 0.8 Hz), 6.15 and 6.22 (each *dd*, 1H, J = 17.8, 10.4 and 17.0, 10.4 Hz) which would substitute on C-3 and C-8. On the basis of the above results, the structure of claucavatin-A was deduced as **1**.

Claucavatin-B (**2**) was obtained as granules. Its molecular formula, containing two more hydrogen atoms than **1**, was established as $C_{24}H_{30}O_5$. However, the spectral data were very closely related to those of **5** (Table 1). The major difference was the presence of three mutually coupled aliphatic protons at δ 2.08, 2.41 (each 1H, *br d*, J = 15.0 Hz)) and 4.22 (1H, *br s*) which were attributed to two H-4′ and one H-3′, respectively. The downfield signal of the latter (δ 4.22) indicated that the H-3′ proton is connected to an oxygen functionality, the fact that this signal is a broad singlet (actually a doublet of doublet with a small coupling constant to two H-4′) suggests that H-3′ is oriented in toward the equatorial direction. Examining the molecular formula, a hydroxyl group (δ 5.05) must be attached to C-3′ and orientated in the axial direction. Thus, the double bond between C-3′ and C-4′ in **5** is hydrated to an alcohol in **2**. Hence, structure **2** was established for claucavatin-B.

EXPERIMENTAL

General. Mps: uncorr. UV: in MeOH. IR: in KBr. ^{1}H and ^{13}C NMR: in $CDCl_3$ and TMS as internal reference. MS: a direct inlet system.

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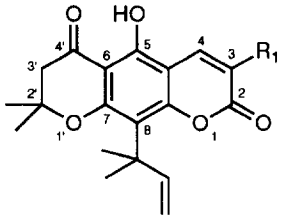
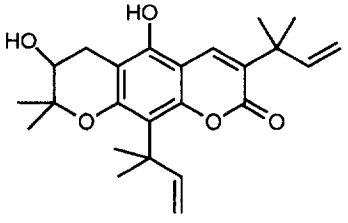
Table 1. ^{1}H NMR spectral data for compounds **1–6** ($CDCl_3$; δ, multiplicity, J (Hz))*

	1	**2**	**3**	**4**	**5**	**6**
H-3 or 3-dimethylallyl	1.46 *s* (2 × CH_3), 4.88 *dd* (10.4, 0.8), 4.92 *dd* (17.8, 0.8), 6.15 *dd* (17.8, 10.4)	1.37 *s* (2 × CH_3), 4.92 *br d* (10.8), 4.99 *br d* (18.5), 6.16 *dd* (18.5, 10.8)	6.15 *d* (9.7)	6.17 *d* (9.8)	1.43 *s* (2 × CH_3), 4.86 *dd* (11.0, 1.1), 4.91 *dd* (17.5, 1.1), 6.18 *dd* (17.5, 11.0)	6.07 *d* (9.7)
H-4	7.89 *s*	7.67 *s*	8.10 *d* (9.7)	8.05 *d* (9.8)	7.85 *s*	8.11 *d* (9.7)
5-OH	13.00 *s*	5.05 *br s*	6.12 *br s*	12.99 *s*	5.69 *s*	8.85 *br s*
8-Dimethylallyl	1.47 s (2 × CH_3), 5.09 *dd* (10.4, 0.8), 5.10 *dd* (17.0, 0.8), 6.22 *dd* (17.0, 10.4)	1.41 *s* (2 × CH_3), 5.06 *br d* (17.0), 5.07 *br d* (11.5), 6.19 *dd* (17.0, 11.5)	1.36 *s* (2 × CH_3), 4.86 *dd* (10.0, 1.0), 4.92 *dd* (17.7, 1.0), 5.92 *dd* (17.7, 10.0)	1.49 *s* (2 × CH_3), 4.89 *dd* (10.4, 1.0), 4.92 *dd* (17.5, 1.0), 6.23 *dd* (17.5, 10.4)	1.47 *s* (2 × CH_3), 5.08 *dd* (11.0, 1.1), 5.09 *dd* (17.5, 1.1), 6.29 *dd* (17.5, 11.0)	1.43 *s* (2 × CH_3), 4.81 *dd* (10.5, 1.3), 4.89 *dd* (17.5, 1.3), 6.30 *dd* (17.5, 10.5)
2′,2′-Dimethyl	1.62 *s*	1.52 *s*	1.63 *s*	1.64 *s*	1.63 *s*	1.63 *s*
H-3′ or 3′-dimethylallyl	2.73 *s*	4.22 *br s*	1.49 *s* (2 × CH_3), 5.07 *dd* (10.0, 1.0), 5.10 *dd* (117.7, 1.0), 6.29 *dd* (17.7, 10.0)	2.76 *s*	5.68 *d* (10.0)	5.73 *d* (10.0)
H-4′	–	2.08, 2.41 *br d* (15.0)	6.47 *s*	–	6.51 *d*	6.75 *d* (10.0)

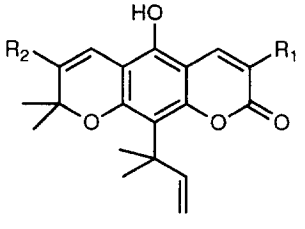
*The assignments for two dimethylallyl g ıay be interchangeable.



1: $R_1 = -C(CH_3)_2CH=CH_2$
4: $R_1 = H$



2



3: $R_1 = H$, $R_2 = -C(CH_3)_2CH=CH_2$
5: $R_1 = -C(CH_3)_2CH=CH_2$, $R_2 = H$
6: $R_1 = H$, $R_2 = H$

Plant material. Clausena excavata was collected from San Dei Men, Pingtung Hsien, Taiwan, in June 1989 and verified by Prof. C. S. Kuoh. The specimen of this plant is deposited in the herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and isolation. The acetone extract of the root bark of *C. excavata* (0.8 kg) was subjected to chromatography on a silica gel column and successively eluted with $CHCl_3$–MeOH and C_6H_6–Me_2CO. Ten coumarins, **1** (19.0 mg), **2** (14.8 mg), **3** (14.8 mg), **4** (7.4 g), **5** (5.2 g), **6** (4.5 g), **7** (8.1 g), **8** (23.7 mg), **9** (15.7 mg) and **10** (1.1 mg), and two flavonoids, **11** (4.2 mg) and **12** (25.8 mg), were found. Several carbazole alkaloids were also found the structures of which have yet to be elucidated.

Claucavatin-A (**1**). Powder, mp 102–104°. HRMS: calcd for $C_{24}H_{28}O_5$, *m/z* 396.1937 $[M]^+$, found 396.1936. UV λ_{max} nm: 201, 219 (sh), 237, 287, 328. IR ν_{max} cm^{-1}: 3450, 1730, 1640. EIMS, *m/z* (rel. int.): 396 (M^+, 100), 381 (93), 369 (11), 353 (17), 325 (54), 299 (15), 149 (67).

Claucavatin-B (**2**). Granules, mp 148–150°. HRMS: calcd for $C_{24}H_{30}O_5$, *m/z* 398.2079 $[M]^+$, found 398.2103. UV λ_{max} nm: 203, 217, 286, 326. IR ν_{max} cm^{-1}: 3450, 1730, 1640, 1630. EIMS, *m/z* (rel. int.): 398 (M^+, 2), 396 (79), 381 (76), 353 (12), 325 (40), 297 (14), 97 (34), 83 (42), 69 (100), 57 (94).

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