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CONSTITUENTS FROM THE STEMS OF *DENDROBIUM CLAVATUM* VAR. *AURANTIACUM*

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Nine compounds were isolated from the stems of *Dendrobium clavatum* var. *aurantiacum*. On the basis of spectral analysis, they were identified as coumarin (**1**), alkyl 4´-hydroxy-*trans*-cinnamates (**2**), campesterol (**3**), stigmasterol (**4**), β-sitosterol (**5**), aliphatic alcohols (**6**), alkyl *trans*-ferulates (**7**), stigmast-4-en-3-one (**8**), and aliphatic acids (**9**). These compounds were first isolated from this plant. Among them, coumarin (**1**) is the major component in both *n*-hexane and ethyl acetate extracts.

Key words: *Dendrobium clavatum* var. *aurantiacum*, Orchidaceae, Coumarin, Alkyl 4´-hydroxy-*trans*-cinnamates, Alkyl *trans*-ferulates, Stigmast-4-en-3-one.

INTRODUCTION

The important Chinese herb "Shi-Hu (石斛)" is prepared from the dried stems of *Dendrobium* species (Orchidaceae) and is used as a tonic and an antipyretic.[1] There are 1600 species of *Dendrobium* in the world [2] and 15 species in Taiwan.[3]

In the course of our studies on the Taiwanese Orchidaceae plants, we earlier reported that the constituents of *Dendrobium moniliforme* (LINNE) SWARTZ, contained a novel compound, 2,6-dimethoxy-1,4,5,8-phenanthradiquinone along with denbinobin, a number of aromatics, long chain fatty acids and esters, and phytosterols, as well as denbinobin showed potent anti-inflammatory effect *in vitro*. [4,5]

However there have been no previous literature reports on the constituents of *D. clavatum* LINDLEY var. *aurantiacum* (REICHENBACH f.) TANG et WANG. In this paper, we report the isolation and structure elucidation of nine compounds from the title plant.

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MATERIALS AND METHODS

General

Melting points were determined on a Yanaco MP-500D micromelting point apparatus and were uncorrected. IR spectra were recorded on a Nicolet Impact 400 FT-IR spectrometer. UV spectra were measured on a Shimadzu UV-160A recording spectrometer. Mass spectra were taken on a JEOL JMS-SX/SX 102A tandem mass spectrometer. NMR spectra were run on either a Varian Unity VXR-300 FT NMR or Bruker DPX-200, DMX-500 FT NMR spectrometers. Chemical shifts are given in δ-values and coupling constants (*J*) are given in hertz (Hz). GC-MS analysis were performed on a Hewlett Packard 5995 GC-MS system. A HP-20, 25 m × 0.32 mm (i.d.) capillary column was used for GC-MS. The column temperature was programmed from 110℃ to 300℃ at a rate of 5℃/min. Helium was used as the carrier gas at a flow-rate of 1 mL/min.

Plant Material

The stems of *D. clavatum* var. *aurantiacum* was collected in Chiayi Hsien, Taiwan during July 1995 and was identified by Professor Chung-Chuan Chen of the Institute of Chinese Pharmaceutical Sciences, China Medical College, Taichung, Taiwan. A voucher specimen (No. CMC-1901-DCA-1) is maintained in the herbarium of this institute.

Extraction and Isolation

The air-dried stems of *D. clavatum* var. *aurantiacum* were chopped into small pieces (2 kg) and successively extracted with *n*-hexane (2 L × 4) and EtOAc (2 L × 4) at room temperature to afford *n*-hexane extract (12 g) and EtOAc extract (85 g). Both the *n*-hexane and EtOAc extracts were chromatographed on silica gel and eluted with a stepwise gradient mixture of *n*-hexane-EtOAc, followed by Sephadex LH-20 column using chloroform-methanol (1:1) as elutent. Five and nine compounds were obtained from *n*-hexane and EtOAc extracts respectively. Compounds **1** (2.84 g), **2** (0.15 g), and a mixture of **3** to **5** (0.11 g) from the *n*-hexane extract. Compounds **1** (7.43 g), **2** (0.15 g), a mixture of **3** to **5** (0.10 g), **6** (0.15 g), **7** (41.0 mg), **8** (10.0 mg), and **9** (0.38 g) from the EtOAc extract.

Coumarin (1)

White needles from *n*-hexane, mp. 66-67℃; EIMS *m/z* (%) (rel. int.): 146 $[M]^+$ (90), 118 (100), 90 (48), 89 (58) and 63 (54) ; IR (KBr) cm^{-1}: 2919, 2849, 1712, 1607, 1455, 1403, 1177, 1123, 835 and 758; UV λ_{max} nm ($CHCl_3$) (log ε): 274 (4.03), 313 (3.74); ^1H NMR ($CDCl_3$, 500 MHz): δ 6.40 (1H, *d*, *J* = 9.5 Hz, H-3), 7.26 (1H, *br dd*, *J* = 7.7 and 7.7 Hz, H-6), 7.31 (1H, *br d*, *J* = 8.3 Hz, H-8), 7.46 (1H, *dd*, *J* = 7.7 and 1.5 Hz, H-5), 7.51 (1H, *ddd*, *J* = 8.3, 7.7 and 1.5 Hz, H-7), 7.69 (1H, *d*, *J* = 9.5 Hz, H-4); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 116.7 (C-3), 116.9 (C-8), 118.8 (C-4a), 124.4 (C-6), 127.9 (C-7), 131.8 (C-5), 143.4 (C-4), 154.1 (C-8a) and 160.8 (C-2).

Alkyl 4´-hydroxy-*trans*-cinnamates (2)
[*n*-hexacosyl and *n*-octacosyl 4´-hydroxy-*trans*-cinnamates are the major components]

White solid from chloroform, mp. 73-75°C; EIMS *m/z* (%) (rel. int.): 612 $[M]^+$ (0.1) (*n*-dotriaconyl 4´-hydroxy-*trans*-cinnamate), 598 $[M]^+$ (0.1) (*n*-hentriaconyl 4´-hydroxy-*trans*-cinnamate), 584 $[M]^+$ (3) (*n*-triaconyl 4´-hydroxy-*trans*-cinnamate), 570 $[M]^+$ (0.9) (*n*-nonacosyl 4´-hydroxy-*trans*-cinnamate), 556 $[M]^+$ (24) (*n*-octacosyl 4´-hydroxy-*trans*-cinnamate), 542 $[M]^+$ (3) (*n*-heptacosyl 4´-hydroxy-*trans*-cinnamate), 528 $[M]^+$ (33) (*n*-hexacosyl 4´-hydroxy-*trans*-cinnamate), 514 $[M]^+$ (3) (*n*-pentacosyl 4´-hydroxy-*trans*-cinnamate), 500 $[M]^+$ (9) (*n*-tetracosyl 4´-hydroxy-*trans*-cinnamate), 486 $[M]^+$ (0.5) (*n*-tricosyl 4´-hydroxy-*trans*-cinnamate), 472 $[M]^+$ (1) (*n*-docosyl 4´-hydroxy-*trans*-cinnamate), 164 (100), 147 (61), 120 (22), 107 (17), 91 (7), 71 (14), 57 (33), 43 (37); IR (KBr) cm^{-1}: 3375, 2912, 2849, 1718, 1607, 1516, 1462, 1172 and 835; UV λ_{max} nm ($CHCl_3$): 299, 248 (sh); ^1H NMR ($CDCl_3$): δ 0.87 (3H, *t*, *J* = 6.6 Hz, CH_3), 1.24 (*br s*, $(CH_2)_n$), 1.67 (2H, *t*, *J* = 6.3 Hz, $OCH_2C\underline{H}_2$), 4.18 (2H, *t*, *J* = 6.7 Hz, OCH_2), 5.44 (1H, *br s*, OH), 6.29 (1H, *d*, *J* = 15.9 Hz, H-2), 6.83 (2H, *d*, *J* = 8.6 Hz, H-3´ and H-5´), 7.42 (2H, *d*, *J* = 8.6 Hz, H-2´ and H-6´) and 7.61 (1H, *d*, *J* = 15.9 Hz, H-3) ; ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3), 22.7, 26.0, 29.3, 29.4, 29.7, 31.9 ($(CH_2)_n$), 28.7 ($OCH_2\underline{C}H_2$), 64.7 (OCH_2), 115.6 (C-2), 115.9 (C-3´ and C-5´), 127.2 (C-1´), 129.9 (C-2´ and C-6´), 144.4 (C-3), 157.8 (C-4´) and 167.7 (C-1).

Phytosterol [mixture of campesterol (3), stigmasterol (4) and β-sitosterol (5)]

White crystals from chloroform, mp. 138-140°C; EIMS *m/z* (%) (rel. int.): 414 $[M]^+$ (100), 412 $[M]^+$ (26), 400 $[M]^+$ (21), 369 (30), 255 (24), 213 (18), 145 (21), 81 (37), 69 (55), 55 (38), 43 (47), 41 (28); IR (KBr) cm^{-1}: 3418, 2940, 2865, 1460, 1375, 1053; ^1H NMR ($CDCl_3$): δ 0.66 (3H, *s*, H-18), 0.80 (3H, *t*, *J* = 6.8 Hz, H-29), 0.81 (3H, *d*, *J* = 6.8 Hz, H-26), 0.81 (3H, *d*, *J* = 6.8 Hz, H-27), 0.90 (3H, *d*, *J* = 6.4 Hz, H-21), 0.99 (3H, *s*, H-19), 3.49 (1H, *m*, H-3α), 4.98 (1H, *dd*, *J* = 15.1 and 8.2 Hz, H-22), 5.14 (1H, *dd*, *J* = 15.1 and 8.2 Hz, H-23), 5.33 (1H, *d*, *J* = 5.2 Hz, H-6).

GC-MS analysis exhibited the molecular ion peak at *m/z* 400, 412 and 414, which indicated that this fraction contained a mixture of campesterol (**4**, t_R = 24.6 min, *m/z* 400 $[M]^+$), stigmasterol (**5**, t_R =25.6 min, *m/z* 412 $[M]^+$) and β-sitosterol (**6**, t_R =27.4 min, *m/z* 414 $[M]^+$).

Aliphatic alcohols (6)

White solid from chloroform, mp. 76-78°C; EIMS *m/z* (%) (rel. int.): 476 $[M-H_2O]^+$ (3), 448 $[M-H_2O]^+$ (24), 434 $[M-H_2O]^+$ (8), 420 $[M-H_2O]^+$ (17), 406 $[M-H_2O]^+$ (5), 392 $[M-H_2O]^+$ (83), 378 $[M-H_2O]^+$ (9), 364 $[M-H_2O]^+$ (46), 350 $[M-H_2O]^+$ (5) and 336 $[M-H_2O]^+$ (12); IR (KBr) cm^{-1}: 3298, 2925, 2849, 1467, 1072 and 736; ^1H NMR ($CDCl_3$):δ 0.86 (3H, *t*, *J* = 6.4Hz), 1.23 (*br.s*), 1.56 (2H, *m*), 3.62 (2H, *t*, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$):δ 14.1, 22.6, 25.7, 29.4, 29.6, 31.9, 32.8 and 63.1.

Alkyl *trans*-ferulates (7)

[*n*-tetracosyl and *n*-tricosyl *trans*-ferulates are major components]

White solid from chloroform, mp. 57-59°C; EIMS *m/z* (%) (rel. int.): 614 $[M]^+$ (0.3) (*n*-triaconyl *trans*-ferulate), 586 $[M]^+$ (0.2) (*n*-octacosyl *trans*-ferulate), 572 $[M]^+$ (2) (*n*-heptacosyl *trans*-ferulate), 558 $[M]^+$ (12) (*n*-hexacosyl *trans*-ferulate), 544 $[M]^+$ (0.6) (*n*-pentacosyl *trans*-ferulate), 530 $[M]^+$ (66) (*n*-tetracosyl *trans*-ferulate), 516 $[M]^+$ (35) (*n*-tricosyl *trans*-ferulate), 502 $[M]^+$ (17) (*n*-docosyl *trans*-ferulate), 488 $[M]^+$ (18) (*n*-heneicosyl *trans*-ferulate), 474 $[M]^+$ (18) (*n*-eicosyl *trans*-ferulate), 460 $[M]^+$ (1) (*n*-nonadecyl *trans*-ferulate), 446 $[M]^+$ (5) (*n*-octadecyl *trans*-ferulate), 194 (100), 177 (91), 57 (52), 43 (68); IR (KBr) cm^{-1}: 3540, 2919, 2849, 1712, 1681, 1600, 1523, 1172 and 1039; UV λ_{max} nm ($CHCl_3$): 320 and 243; ^1H NMR ($CDCl_3$): δ 0.86 (3H, *t*, *J* = 6.7 Hz, CH_3), 1.23 (*br s*, $(CH_2)_n$), 1.67 (2H, *t*, *J* = 6.6 Hz, $OCH_2C\underline{H}_2$), 3.91 (3H, *br s*, OCH_3), 4.17 (2H, *t*, *J* = 6.7 Hz, OCH_2), 5.86 (1H, *br s*, OH), 6.27 (1H, *d*, *J* = 15.9 Hz, H-2), 6.89 (1H, *d*, *J* = 8.0 Hz, H-5´), 7.03 (1H, *d*, *J* = 2.6 Hz, H-2´), 7.05 (1H, *dd*, *J* = 8.0, 2.6 Hz, H-6´) and 7.59 (1H, *d*, *J* = 15.9 Hz, H-3) ; ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3), 22.7, 26.0, 29.4, 29.7, 31.9 ($(CH_2)_n$), 28.8 ($OCH_2\underline{C}H_2$), 55.9 ($O\underline{C}H_3$), 64.6 ($O\underline{C}H_2$), 109.3 (C-2´), 114.7 (C-5´), 115.7 (C-2), 123.0 (C-6´), 127.1 (C-1´), 144.6 (C-3), 146.8 (C-4´), 147.9 (C-3´) and 167.4 (C-1).

Stigmast-4-en-3-one (8)

White needles from *n*-hexane, mp. 87-89°C; EIMS *m/z* (%) (rel. int.): 412 $[M]^+$ (100), 370 (8), 289 (12), 271 (10), 229 (31), 147 (29) and 124 (93) ; IR (KBr) cm^{-1}: 2926, 2856, 1677, 1621, 1469 and 1392; UV λ_{max} nm ($CHCl_3$) (log ε): 246 (4.20); ^1H NMR ($CDCl_3$): δ 0.68 (3H, *s*, H-18), 0.78 (3H, *d*, *J* = 6.8 Hz, H-27), 0.80 (3H, *d*, *J* = 6.8 Hz, H-26), 0.81 (3H, *t*, *J* = 6.8 Hz, H-29), 0.89 (3H, *d*, *J* = 6.5 Hz, H-21), 1.15 (3H, *s*, H-19), 2.34 (2H, *m*, H-2), 5.70 (1H, *s*, H-4) ; ^{13}C NMR ($CDCl_3$): δ 11.9 (C-18), 12.0 (C-29), 17.3 (C-19), 18.6 (C-21), 18.9 (C-27), 19.8 (C-26), 21.0 (C-11), 23.0 (C-28), 24.1(C-15), 26.0 (C-23), 28.1 (C-16), 29.1 (C-25), 32.0 (C-2), 32.9 (C-7), 33.8 (C-6), 33.9 (C-22), 35.5 (C-8), 35.6 (C-1), 36.1 (C-20), 38.5 (C-10), 39.6 (C-12), 42.3 (C-13), 45.8 (C-24), 53.8 (C-9), 55.8 (C-17), 55.9 (C-14), 123.7 (C-4), 171.7 (C-5) and 199.6 (C-3).

Aliphatic Acids (9)

White solid from acetone, mp. 78-79°C; EIMS *m/z* (%) (rel. int.): 508 (0.2), 494 (0.2), 480 (3), 466 (1), 452 (18), 438 (6), 424 (86), 410 (15), 396 (67), 382 (23), 368 (62), 354 (22), 340 (17), 241 (9), 185 (19), 97 (52), 57 (100) and 43 (82); IR (KBr) cm^{-1}: 3460, 2919, 2844, 1712 and 1474; ^1H NMR ($CDCl_3$):δ 0.86 (3H, *t*, *J* = 6.5Hz), 1.23 (*br.s*), 1.59 (2H, *m*), 2.33 (2H, *t*, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$):δ 14.1, 22.7, 24.7, 29.7, 31.9, 33.6 and 177.3.

RESULTS AND DISCUSSIONS

Nine compounds were isolated from the stems of *D. clavatum* var. *aurantiacum*. Compounds **1** showed a molecular peak at m/z 146 in the EIMS. IR absorptions at 1712 cm^{-1} supported for presence of a carbonyl group, 1607 and 1455 cm^{-1} indicated an aromatic system. ^{1}H NMR spectrum indicated the presence of four aromatic protons at δ 7.26 (1H, *br dd*, *J* = 7.7 and 7.7 Hz, H-6), 7.31 (1H, *br d*, *J* = 8.3, H-8), 7.46 (1H, *dd*, *J* = 7.7 and 1.5 Hz, H-5), 7.51 (1H, *ddd*, *J* = 8.3, 7.7, and 1.5 Hz, H-7), and two olefinic protons at δ 6.40 (1H, *d*, *J* = 9.5 Hz, H-3), 7.69 (1H, *d*, *J* = 9.5 Hz, H-4). Furthermore, the 2D NMR (^{1}H-^{1}H COSY, HMQC, HMBC and NOESY) data permitted complete assignments for ^{1}H and ^{13}C-NMR chemical shifts of this compound. Herein, compound **1** was identified as coumarin on the basis of the above data and comparision of its spectral data with literature values.[6,7]

Compounds **2**, **3**, **4**, **5**, **7**, and **8** which were identified as alkyl 4´- hydroxy-*trans*-cinnamates,[8-11] campesterol, stigmasterol, β-sitosterol, alkyl *trans*-ferulates,[11-14] and stigmast-4-en-3-one,[15] respectively, which we also already isolated from *D. moniliforme*.[5] The coupling constant between H-2 and H-3 (*J* = 15.9 Hz) clearly suggested *trans*-geometry of compound **2** and **7**. The compositions of compound **2** and **7** were roughly suggested by EIMS and revealed to be a mixture of C_{22}-C_{32} alkyl cinnamates and a mixture of C_{18}-C_{28}, C_{30} alkyl ferulates, respectivey. Alkyl 4´-hydroxy-*trans*-cinnamates (**2**) contain 11 components including *n*-dotriaconyl, *n*-hentriaconyl, *n*-triaconyl, *n*-nonacosyl, *n*-octacosyl, *n*-heptacosyl, *n*-hexacosyl, *n*-pentacosyl, *n*-tetracosyl, *n*-tricosyl, and *n*-docosyl 4´-hydroxy-*trans*-cinnamates, among which *n*-hexacosyl and *n*-octacosyl 4´-hydroxy-*trans*-cinnamates are major components. Alkyl *trans*-ferulates (**7**) contain 12 components including *n*-triaconyl, *n*-octacosyl, *n*-heptacosyl, *n*-hexacosyl, *n*-pentacosyl, *n*-tetracosyl, *n*-tricosyl, *n*-docosyl, *n*-heneicosyl, *n*-eicosyl, *n*-nonadecyl, and *n*-octadecyl *trans*-ferulates, among them, *n*-tetracosyl and *n*-tricosyl *trans*-ferulates are major components. Compound **6** and **9** were identified as aliphatic alcohols and aliphatic acids by spectral analysis.

These compounds were first isolated from this plant and coumarin (**1**) is the major component.

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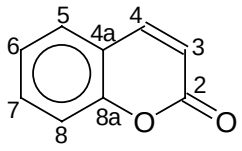

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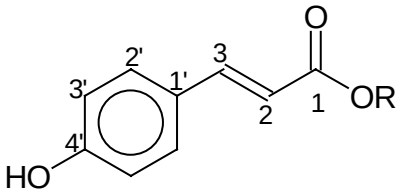
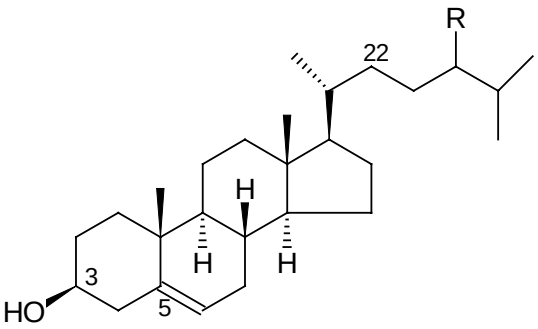


2 R = C_nH_{2n+1}, n = 22-32



$CH_3(CH_2)_nCH_2R$

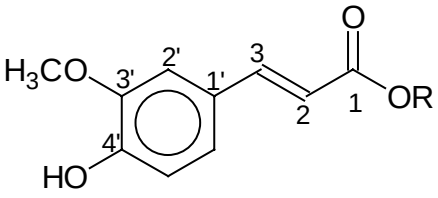
3 R = CH_3

4 R = C_2H_5 , Δ^{22}

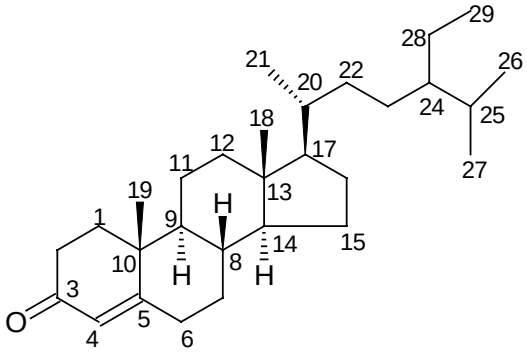
5 R = C_2H_5

6 R = OH, n = 22-32

9 R= COOH, n = 19-31



7 R = C_nH_{2n+1}, n = 18-28, 30



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金草蘭(*Dendrobium clavatum* var. *aurantiacum*)莖之成分研究

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從金草蘭莖部分離得到 9 個化合物，經由光譜分析，鑑定為：coumarin (**1**)，alkyl 4´-hydroxy-*trans*-cinnamates (**2**)，campesterol (**3**)，stigmasterol (**4**)，β-sitosterol (**5**)，aliphatic alcohols (**6**)，alkyl *trans*-ferulates (**7**), stigmast-4-en-3-one (**8**)，及 aliphatic acids (**9**)，這些化合物是首次自本植物分離。在這些化合物中，coumarin (**1**)為正己烷及乙酸乙酯抽出物之主成分。

關鍵詞：金草蘭，蘭科，Coumarin，Alkyl 4´-hydroxy-*trans*-cinnamates，Alkyl *trans*-ferulates，Stigmast-4-en-3-one。

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