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MONO-AROMATIC CONSTITUENTS OF *Dendrobium longicornu*

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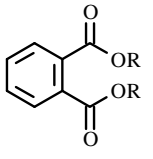
The stems of several *Dendrobium* species (Orchidaceae) are used in traditional Chinese medicine as a tonic to nourish the stomach, promote the production of body fluid, and reduce fever [1]. The products prepared from the dried stems of *Dendrobium* plants are also used as precious health foods and nutrients [2]. Earlier work on the genus led to the isolation of a series of compounds such as alkaloids, fluorenones, sesquiterpenoids, bibenzyls, and phenanthrenes with antioxidant, antitumor, antimutagenic, and other activities [3–6]. *D. longicornu* Lindl is distributed in Nepal, Sikkim, Bhutan, India, Vietnam, and the southwestern part of China [7]. Previously, there have been no reports on its chemical constituents. In the course of our search for new bioactive natural products from medicinal plants in Yunnan of China, we investigated the plant.

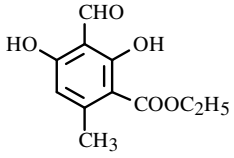
D. longicornu was collected from Lianghe County of Yunnan Province, China in September, 2004. The air-dried whole plants (3.4 kg) were chopped and extracted with 95% EtOH five times (each 20 L) at room temperature. The EtOH extract (115 g) was diluted with H_2O (1 L) and then extracted with petroleum ether, EtOAc, and *n*-BuOH (each 0.5 L, five times) successively. Evaporation of the respective solvents gave the petroleum ether (30 g), EtOAc (26 g), and *n*-BuOH (52 g) extracts.

The EtOAc extract (26 g) was applied to a silica gel column (200–300 mesh, 100 cm × 10 cm), eluting with petroleum ether containing increasing amounts of acetone to obtain six fractions monitored by TLC tests. Spots on the plate were observed under UV light and visualized by spraying with 15% H_2SO_4 in ethanol followed by heating. Fraction 1 (2 g) was further purified on column chromatography (silica gel, 200–300 mesh, 60 cm × 4 cm, petroleum ether–acetone 30:1) to afford five subfractions. The second and third subfractions were isolated on preparative TLC (20 cm × 20 cm, petroleum ether–benzene 1:1) to afford **1** (40 mg) and **2** (35 mg), respectively. The fourth subfraction was purified by recrystallization from acetone to obtain **8** (100 mg); Fr. 2 (4.5 g) was separated on column chromatography (silica gel, 200–300 mesh, 60 cm × 5 cm, $CHCl_3$–acetone 15:1) to yield five subfractions. The second subfraction was isolated on a Sephadex LH-20 column (120 cm × 3 cm, MeOH) to obtain **5** (15 mg). The third subfraction was purified on a preparative TLC plate (20 cm × 20 cm, petroleum ether–EtOAc 9:1) to afford **7** (8 mg). The fourth subfraction was separated on a preparative TLC plate (20 cm × 20 cm, $CHCl_3$–acetone 15:1) to afford **6** (6 mg); Fr. 5 (9 g) was purified on column chromatography (silica gel, 200–300 mesh, 60 cm × 4 cm, $CHCl_3$–acetone 10:1) to afford two subfractions. The second subfraction (3 g) was subjected to repeated column chromatography similarly, first on Sephadex LH-20 (MeOH), and then on preparative TLC (20 cm × 20 cm, petroleum ether–EtOAc 2:1, $CHCl_3$–MeOH 15:1) to isolate **3** (11 mg) and **4** (20 mg).

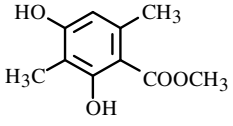


1, 2

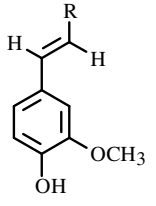
1: R = $CH_2CH(C_2H_5)(CH_2)_3CH_3$
2: R = $(CH_2)_3CH_3$



3



4



5,7

5: R = $COOCH_2(CH_2)_{20}CH_3$
7: R = CHO

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Compound **1**, $C_{24}H_{38}O_4$, yellow oil. The mass spectrum exhibited peaks for ions at *m/z* 390 $[M]^+$, 179, 167, 149 (100), 113, 71, 57. The PMR spectrum ($CDCl_3$, δ, ppm, J/Hz) displayed characteristic aromatic proton signals of phthalic acid ester [7.73 (2H, dd, J = 7.0, 3.7, H-3, 6), 7.54 (2H, dd, J = 7.0, 3.7, H-4, 5)] and two similar ester moieties [4.31 (4H, t, J = 6.0, H-1′), 1.74 (2H, m, H-2′), 1.47 (4H, m, H-1″), 1.38 (4H, m, H-5′), 1.35 (4H, m, H-3′), 1.31 (4H, m, H-4′), 0.91 (6H, t, J = 7.5, H-2″), 0.87 (6H, t, J = 7.0, H-6′)]. The ^{13}C NMR and DEPT spectra ($CDCl_3$, δ, ppm) had signals at 167.7 (COO-), 132.4 (C-1,2), 130.9 (C-4,5), 128.8 (C-3,6), 68.1 (C-1′), 38.7 (C-2′), 30.4 (C-3′), 28.9 (C-4′), 23.7 (C-1″), 23.0 (C-5′), 14.0 (C-6′), 10.9 (C-2″). Based on NMR, including 2D NMR (H-H COSY, C-H COSY, HMQC, and HMBC), and mass spectral data, and comparison of the spectral data with those reported in literature, **1** was identified as bis(2-ethylhexyl)phthalate [8].

Compound **2**, $C_{16}H_{22}O_4$, colorless oil. The mass spectrum exhibited peaks for ions at *m/z* 278 $[M]^+$, 149 (100). The PMR spectrum ($CDCl_3$, δ, ppm, J/Hz) also showed characteristic aromatic proton signals of phthalic acid ester [7.70 (2H, dd, J = 7.0, 3.7, H-3, 6), 7.50 (2H, dd, J = 7.0, 3.7, H-4, 5)] and two similar ester moieties [4.29 (4H, t, J = 6.5, H-1′), 1.70 (2H, m, H-2′), 1.42 (4H, sextet, J = 7.5, H-3′), 0.96 (6H, t, J= 7.5, H-4′). The ^{13}C NMR and DEPT spectra ($CDCl_3$, δ, ppm) had signals at 167.7 (COO-), 132.3 (C-1,2), 130.9 (C-4,5), 128.8 (C-3,6), 65.6 (C-1′), 30.6 (C-2′), 19.2 (C-3′), 13.7 (C-4′). Based on NMR and mass spectral data, and comparison of the spectral data with those reported in literature, **2** was identified as dibutyl phthalate [8].

Compound **3**, $C_{11}H_{12}O_5$, colorless crystal, mp 110–112°C. The mass spectrum exhibited peaks for ions at *m/z* 224 $[M]^+$, 196, 179, 178, 177, 168, 150 (100). The PMR spectrum ($CDCl_3$, δ, ppm, J/Hz) displayed an aromatic proton [6.28 (1H, s, H-5)], an aromatic methyl [2.54 (3H, s, CH_3-6)], an aldehyde group [10.34 (1H, s, CHO)], two hydroxys [12.96 (1H, s, HO-2), 12.39 (1H, s, HO-4)], and an ethoxyl [4.25 (2H, q, J = 7.1), 1.43 (3H, t, J = 7.1)]. The ^{13}C NMR and DEPT spectra ($CDCl_3$, δ, ppm) had signals at 193.9 (-CHO), 171.6 (COO-), 168.4 (C-2), 166.6 (C-4), 152.4 (C-6), 112.1 (C-5), 108.6 (C-3), 104.0 (C-1), 61.8 (OCH_2CH_3), 25.2 (CH_3-6), 14.2 (OCH_2CH_3)]. Based on NMR, including 2D NMR (C-H COSY, HMQC, HMBC, and NOESY), and mass spectral data, and comparison of the spectral data with those reported in literature, **3** was identified as ethyl haematommate [9].

Compound **4**, $C_{10}H_{12}O_4$, colorless crystal, mp 137–139°C. The mass spectrum exhibited peaks for ions at *m/z* 196 $[M]^+$, 155, 127, 111, 97, 71, 57 (100). The PMR spectrum ($CDCl_3$, δ, ppm, J/Hz) indicated an aromatic proton [6.24 (1H, s, H-5)], two aromatic methyls [2.49 (3H, s, CH_3-3), 2.14 (3H, s, CH_3-6)] and a methoxyl [3.95 (3H, s)]. The ^{13}C NMR and DEPT spectra ($CDCl_3$, δ, ppm) had signals at 173.5 (COO), 164.1 (C-4), 158.9 (C-2), 141.0 (C-6), 111.4 (C-5), 109.4 (C-3), 106.2 (C-1), 52.6 (OMe), 24.9 (CH_3-6), 8.5 (CH_3-3)]. Based on NMR, including 2D NMR (C-H COSY, HMQC, HMBC, and NOESY), and mass spectral data, and comparison of the spectral data with those reported in literature, **4** was identified as methyl *β*-orcinol carboxylate [10].

Compound **5**, $C_{32}H_{54}O_4$, white powder. The mass spectrum exhibited peaks for ions at *m/z* 502 $[M]^+$ (100), 488, 474, 194, 97, 177. The PMR spectrum ($CDCl_3$, δ, ppm, J/Hz) showed a 1,3,4-trisubstitued benzene ring [7.11 (1H, dd, J = 8.1, 2.0, H-6), 6.91 (1H, d, J = 8.0, H-5), 7.07 (1H, d, J = 2.0, H-2)], a pair of *trans*-alkene protons [7.65 (1H, d, J = 15.9, H-7), 6.32 (1H, d, J = 15.9, H-8)], a methoxyl [3.95 (3H, s)], and a long-chain alkoxyl group [4.22 (2H, t, J = 6.7, $OC\underline{H}_2CH_2$), 1.74 (2H, t, J = 6.7, $OCH_2C\underline{H}_2$), 1.29 (br.s, $(CH_2)_n$), 0.91 (3H, t, J = 6.7, CH_3)]. The ^{13}C NMR and DEPT spectra ($CDCl_3$, δ, ppm) showed signals at 168.2 (COO-), 148.8 (C-3), 147.7 (C-4), 145.5 (C-7), 127.9 (C-1), 123.9 (C-6), 116.3 (C-8), 115.6 (C-5), 110.2 (C-2), 65.5 (OCH_2), 56.8 (OMe-3), 32.8, 30.6, 30.2, 29.7, 29.6, 26.9, 23.6 (CH_2), 15.0 (CH_3). Based on NMR and mass spectral data, and comparison of the spectral data with those reported in literature, **5** was identified as *n*-docosyl *trans*-ferulate [11].

Compound **6**, $C_7H_6O_5$, white neddles, mp 248–250°C. The ESI mass spectrum exhibited peaks for ions at *m/z* 169 $[M-H]^-$. The PMR spectrum (CD_3OD, δ, ppm, J/Hz) showed only one aromatic proton signal [7.09 (2H, s, H-2,6). The ^{13}C NMR and DEPT spectra (CD_3OD, δ, ppm) had signals at 171.0 (COOH), 146.8(C-3,5), 140.0 (C-4), 122.6 (C-1), 110.8 (C-2,6). By comparison of the spectral data with those reported in literature, **6** was identified as gallic acid [12].

Compound **7**, $C_{10}H_{10}O_3$, yellow oil. The ESI mass spectrum exhibited peaks for ions at *m/z* 177 $[M-H]^-$. The PMR spectrum (CD_3OD, δ, ppm, J/Hz) showed a 1,3,4-trisubstitued benzene ring [7.10 (1H, dd, J = 8.0, 2.0, H-6), 7.08 (1H, d, J = 2.0, H-2), 6.69 (1H, d, J = 8.0, H-5)], a pair of *trans*-alkene protons [7.54 (1H, d, J = 15.3, H-7), 6.51 (1H, dd, J = 15.3, 8.0, H-8)], a methoxyl [3.84 (3H, s)], and an aldehyde group [9.44 (1H, d, J = 8.0, CHO)]. The ^{13}C NMR and DEPT spectra (CD_3OD, δ, ppm) had signals at 196.1 (CHO), 156.7 (C-7), 154.5 (C-4), 150.2 (C-3), 126.6 (C-1), 125.8 (C-8), 125.7 (C-6), 117.2 (C-5), 112.1 (C-2), 56.5 (OCH_3). By comparison of the spectral data with those reported in literature, 7 was identified as ferulaldehyde [13].

Compound **8**, $C_{29}H_{50}O$, was identified as *β*-sitosterol. All the compounds were isolated from the plant for the first time.

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