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FLAVONOID AND OTHER COMPOUNDS FROM *Holotrichia diomphalia* LARVAE

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Holotrichia diomphalia larvae is a traditional crude drug in China. In agricultural production, *H. diomphalia* larvae is a pest to field crops but has significant physiological activity on arthrolithiasis, tetanus, erysipelas, and superficial infection [1]. These grubs are one of the most widely used folk medicinal preparations in Korea for treatment of chronic liver cirrhosis, contusion, edema, furuncle, and apoplexy [2]. We studied previously the composition of the petroleum ether extracts of *H. diomphalia* larvae using GC-MS [3, 4]. However, compounds of the different classes were not well separated from the insect larvae. Herein we report for the first time four known compounds, a flavonoid and three other compounds, isolated from the extract of *H. diomphalia* larvae by ethyl acetate and petroleum ether.

H. diomphalia larvae was purchased at an herbal drug market in Shanghai, China in November 2006 and identified by Prof. H. M. Zhang, A voucher specimen has been deposited in the School of Pharmacy, Second Military Medical University, Shanghai, China.

Torrefied (50°C) and chopped *H. diomphalia* larvae were refluxed three times (3 h each time) with 75% ethanol. The materials were filtered, and the clear supernatant was then concentrated under reduced pressure at 60°C with a vacuum rotary evaporator. The residue was partitioned between water and petroleum ether (60–90°C), EtOAc, and *n*-BuOH, successively. The EtOAc fraction (40g) was subjected to silica gel column chromatography, which was eluted with petroleum ether–ethyl acetate (100:0, 80:1, 50:1, 30:1, 20:1, 10:1, 5:1, 1:1, 0:100, v/v) to form fractions A-I. Fraction I (2.3 g) eluted with ethyl acetate (100%) was chromatographed on a silica gel column (chloroform–methanol, 80:1) and then recrystallized to obtain compound **1** (8.6 mg). Fraction G (15.8 g) eluted with petroleum ether–ethyl acetate 5:1 was further purified by repeated column chromatography (silica gel; petroleum ether–ethyl acetate 50:1 (v/v), petroleum ether–ethyl acetate 30:1 (v/v), and petroleum ether–ethyl acetate 15:1 (v/v)) to afford compounds **2** (30 mg) and **3** (17 mg). A part of the concentrated petroleum ether extract was subjected to repeated chromatography to obtain compound **4** (40.2 mg).

Flavonoids are polyphenols, which are widely distributed in vegetables, fruit, and beverages such as tea and wine. Approximately 5000 flavonoids have been described to date in the plant kingdom [5]; however, only a few have been reported in the literature in the animal kingdom.

The isolated compounds were identified by spectral analysis using MS and NMR spectrometers and by physical chemistry identification (Lieberman-Burchard, HCl–Mg, and $AlCl_3$ reagent), and were determined as tricin (**1**), cholesterol (**2**), palmitinic acid (**3**), and eicosane (**4**) by comparing the data with those in the literature. To the best of our knowledge, compounds **1**–**3** were isolated from the EtOAc extract and compound **4** from the petroleum ether extract of medicinal insects for the first time.

Compound **1**: yellow needle crystal, mp 279–281°C. EI-MS *m/z*: 329.31 $[M - H]^+$, positive reaction with HCl–Mg and $AlCl_3$ reagent, respectively. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.87 (6H, s, C3′ and C5′-OCH_3), 6.20 (1H, d, J = 1.2, H-6), 6.55 (1H, d, J = 1.2, H-8), 6.97 (1H, s, H-3), 7.31 (2H, s, H-2′, 6′), and 12.95 (1H, s, 5-OH). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 56.4 (C3′ and C5′-OCH_3), 94.2 (C-8), 98.8 (C-6), 103.6 (C-3), 103.7 (C-10), 104.4 (C-2′, 6′), 120.4 (C-1′), 139.9 (C-4′), 148.2 (C-3′, 5′), 157.3 (C-9), 161.4 (C-5), 163.6 (C-7), 164.1 (C-2), 181.8 (C-4) [6, 7].

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Compound **2**: white needle crystal, mp 147–149°C. EI-MS *m/z*: 387 $[M + 1]^+$, positive reaction with Lieberman-Burchard reagent. ^1H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.733 (3H, s, 18-CH_3), 0.863 (3H, d, J = 6.6, 26 or 27-CH_3), 0.852 (3H, d, J = 6.6, 26 or 27-CH_3), 0.914 (3H, d, J = 6.5, 21-CH_3), 1.91 (3H, s, 19-CH_3), 3.454 (1H, m, 3-H), 5.286 (1H, br.d, J = 5.2, 6-H). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 11.86 (C-18), 18.72 (C-21), 19.39 (C-19), 21.09 (C-11), 22.55 (C-26), 22.81 (C-27), 23.83 (C-23), 24.30 (C-15), 28.01 (C-25), 28.23 (C-16), 31.67 (C-2), 31.92 (C-7), 31.92 (C-8), 35.79 (C-20), 36.20 (C-22), 36.51 (C-10), 37.26 (C-1), 39.52 (C-24), 39.80 (C-12), 42.31 (C-4), 42.31 (C-13), 50.15 (C-9), 56.17 (C-17), 56.78 (C-14), 71.81 (C-3), 140.77 (C-5), 121.71 (C-6) [8–11].

Compound **3**: white needle crystal, mp 60–61°C. EI-MS *m/z*: 255.26 $[M - H]^+$. The spectral data agree with those reported in the literature for palmitinic acid [5, 12–14].

Compound **4**: white amorphous powder, mp 36–38°C. EI-MS *m/z*: 281.34 $[M - H]^+$. Spectral data for **4** agree with those reported for eicosane [15].

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