CHEMICAL CONSTITUENTS FROM *Ligularia nanchuanica*

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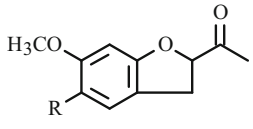
UDC 547.918

Ligularia nanchuanica S. W. Liu, a herbaceous perennial plant belonging to the Compositae family, is one specie of *Ligularia* Cass distributed in Sichuan, Yunnan, and Guizhou provinces of China [1]. As documented in the local pharmacopoeias of these provinces, *L. nanchuanica* is defined as the authentic herbal plants for Chuanziwan, clinically utilized as a replacement for Ziwan with similar antitussive and expectorant actions to those of Ziwan. Up to now, its chemical constituents have not been investigated and there are no reported scientific evidence to support such a substitution [2–4]. In continuing our investigation on bioactive chemical constituents from *Ligularia* Cass, nineteen compounds, including triterpenoids, steroids, benzofuran derivatives, and lignan glycoside, were isolated from an alcoholic extract of the whole plant of *L. nanchuanica.* We report herein the isolation and structural elucidation of all these compounds.

From an alcoholic extract of *L. nanchuanica* S. W. Liu, 2,5-diacetyl-6-methoxybenzofuran (**1**) [5], liguhodgsonal (**2**) [6], ligudentatin (**3**) [6], ergosta-6,22-dien-3β-5α,8α-triol (**4**) [7], ergosta-6-en-3β,5α,8α-triol-22-one (**5**) [7], euparin (**6**) [8], 6-methoxyeuparin (**7**) [9], 5,6-dimethoxy-2-isopropenylbenzofuran (**8**) [5], 5,6-dimethoxy-2-acetylbenzofuran (**9**) [5], caffeic acid (**10**) [10], 7,22-dien-ergosta-3β-ol (**11**) [11], palmitoleic acid-1-monoglycerate (**12**), palmitic acid (**13**) [12], 7,8-dimethoxy-4-methyl-dibenzo-1-carbaldehyde (**14**) [13], lupeol (**15**) [14], 12,29(30)-dien-3β-lupeol (**16**) [15], (+)-4-hydroxypinoresinol-4-*O*-β-D-glucopyranoside (**17**) [16], sucrose (**18**) [17], and β-sitosterol (**19**) were isolated and purified by repeated chromatography over silica gel column. Structures for all these compounds were proposed on the basis of spectroscopic data, together with a comparison of their NMR data with those of the corresponding compounds reported in the literature.

2,5-Diacetyl-6-methoxybenzofuran (1). ^{1}H NMR (400 MHz, $CDCl_3$, TMS, δ, ppm): 3.95 (3H, s, OCH_3), 2.55 (3H, s, CH_3), 2.62 (3H, s, CH_3), 7.45 (1H, s, H-3), 8.06 (1H, s, H-4), 7.07 (1H, s, H-7). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 153.12 (C-2), 114.08 (C-3), 125.82 (C-4), 120.08 (C-5), 158.89 (C-6), 94.82 (C-7), 160.15 (C-8), 127.27 (C-9), 199.13 (C-10), 187.68 (C-11), 26.28 (C-12), 31.74 (C-13), 56.01 (OCH_3).



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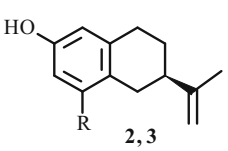

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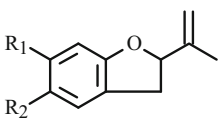

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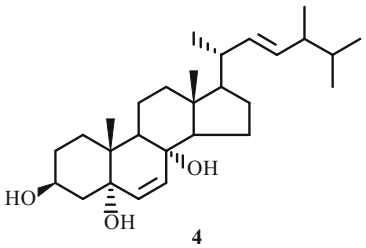

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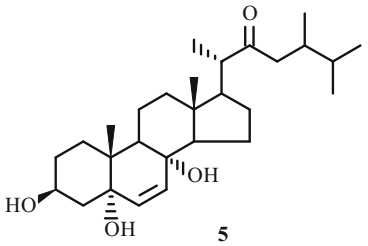
1: R = CH_3CO; **2:** R = CHO; **3:** R = $COOCH_3$; **6:** R_1 = OH, R_2 = $COCH_3$
7: R_1 = OCH_3, R_2 = $COCH_3$; **8:** R_1 = R_2 = OCH_3; **9:** R = OCH_3



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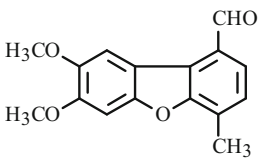

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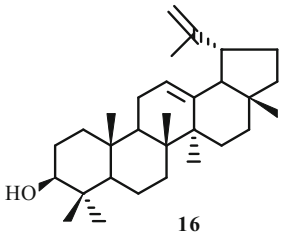

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0009-3130/11/4606-0997

Liguhodgsonal (2). Colorless needle crystals. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 6.85 (1H, J = 2.8, H-1), 7.16 (1H, J = 2.8, H-3), 3.17 (1H, dd, J = 17.3, 4.8, H-6α), 2.77 (1H, dd, J = 17.3, 11.0, H-6β), 2.22 (1H, m, H-7), 1.90 (1H, m, H-8α), 1.62 (1H, m, H-8β), 4.77 (2H, br.s, H-12), 1.80 (3H, br.s, CH_3), 9.76 (1H, s, CHO). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 121.85 (C-1), 153.68 (C-2), 115.77 (C-3), 131.63 (C-4), 134.66 (C-5), 30.27 (C-6), 41.41 (C-7), 27.08 (C-8), 30.27 (C-9), 139.38 (C-10), 148.96 (C-11), 109.26 (C-12), 20.68 (C-13), 192.75 (C-14).

Ligudentatin (3). Colorless needle crystals. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 6.74 (1H, J = 2.8, H-1), 7.14 (1H, J = 2.8, H-3), 3.15 (1H, dd, J = 17.3, 4.8, H-6α), 2.77 (1H, dd, J = 17.3, 11.0, H-6β), 2.22 (1H, m, H-7), 1.90 (m, H-8α), 1.62 (m, H-8β), 4.77 (2 H, br.s, H-12), 1.80 (3H, br.s, CH_3), 3.78 (3H, OCH_3). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 119.50 (C-1), 152.84 (C-2), 115.13 (C-3), 131.00 (C-4), 130.16 (C-5), 32.57 (C-6), 41.77 (C-7), 27.24 (C-8), 30.57 (C-9), 139.79 (C-10), 149.35 (C-11), 109.57 (C-12), 20.68 (C-13), 68.59 (C-14), 52.01 (OCH_3).

Ergosta-6,22-dien-3β,5α,8α-triol (4). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 3.95 (m, H-3), 6.28 (1H, d, J = 8.4, H-6), 6.52 (1H, d, J = 8.4, H-7), 0.82 (3H, s, H-18), 0.88 (3H, s, H-19), 0.90 (3H, d, J = 6.6, H-21), 5.14 (dd, J = 15.4, 8.1, H-22), 5.19 (dd, J = 15.4, 7.3, H-23), 0.83 (3H, d, J = 6.6, H-26), 0.80 (3H, d, J = 6.6, H-27), 0.98 (3H, d, J = 6.6, H-28). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 36.85 (C-1), 30.01 (C-2), 66.36 (C-3), 34.65 (C-4), 79.28 (C-5), 130.57 (C-6), 135.30 (C-7), 82.10 (C-8), 51.03 (C-9), 36.85 (C-10), 20.58 (C-11), 39.29 (C-12), 44.51 (C-13), 51.62 (C-14), 28.59 (C-15), 23.30 (C-16), 56.12 (C-17), 12.62 (C-18), 18.12 (C-19), 39.64 (C-20), 19.40 (C-21), 132.20 (C-22), 135.12 (C-23), 42.70 (C-24), 33.06 (C-25), 19.80 (C-26), 20.63 (C-27), 17.52 (C-28).

Ergosta-6-en-3β,5α,8α-triol-22-one (5). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 3.95 (m, H-3), 6.26 (1H, d, J = 8.4, H-6), 6.50 (1H, d, J = 8.4, H-7), 0.82 (3H, s, H-18), 0.88 (3H, s, H-19), 0.94 (3H, d, J = 6.6, H-21), 0.83 (3H, d, J = 6.6, H-26), 0.80 (3H, d, J = 6.6, H-27), 1.00 (3H, d, J = 6.6, H-28). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 36.85 (C-1), 30.01 (C-2), 66.30 (C-3), 34.65 (C-4), 79.18 (C-5), 130.57 (C-6), 135.30 (C-7), 82.70 (C-8), 51.03 (C-9), 36.85 (C-10), 20.58 (C-11), 39.29 (C-12), 44.51 (C-13), 51.62 (C-14), 28.59 (C-15), 23.30 (C-16), 56.12 (C-17), 12.62 (C-18), 18.12 (C-19), 39.64 (C-20), 19.40 (C-21), 212.62 (C-22), 44.62 (C-23), 42.70 (C-24), 33.06 (C-25), 19.80 (C-26), 20.63 (C-27), 17.52 (C-28).

Euparin (6). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm): 6.95 (1H, H-3), 7.86 (1H, s, H-4), 6.42 (1H, s, H-7), 5.72 (1H, s, H-11a), 5.16 (1H, s, H-11b), 2.60 (3H, s, H-14), 12.48 (6-OH), 2.08 (3H, s, H-12). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 156.12 (C-2), 98.22 (C-3), 120.36 (C-4), 121.24 (C-5), 159.12 (C-6), 102.17 (C-7), 161.12 (C-8), 115.38 (C-9), 131.48 (C-10), 111.26 (C-11), 19.68 (C-12), 202.75 (C-13), 28.45 (C-14).

6-Methoxy-euparin (7). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm): 6.88 (1H, s, H-3), 7.70 (1H, s, H-4), 6.40 (1H, s, H-7), 5.68 (1H, s, H-11a), 5.14 (1H, s, H-11b), 2.05 (3H, s, H-12), 2.60 (3H, s, H-14), 3.93 (3H, s, OMe). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 202.6 (C=O), 157.0 (C-2), 98.9 (C-3), 122.0 (C-4), 120.1 (C-5), 160.2 (C-6), 101.8 (C-7), 161.0 (C-8), 115.7 (C-9), 130.5 (C-10), 111.9 (C-11), 19.0 (C-12), 26.0 (C-14), 56.0 (OMe).

5,6-Dimethoxy-2-isopropenylbenzofuran (8). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm): 6.51 (1H, s, H-3), 7.0 (1H, s, H-4), 6.94 (1H, s, H-7), 5.64 (1H, s, H-11a), 5.06 (1H, s, H-11b), 2.53 (3H, s, H-12), 3.93 (3H, s, OCH_3), 3.90 (3H, s, OCH_3). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 156.10 (C-2), 95.03 (C-3), 120.72 (C-4), 148.16 (C-5), 149.51 (C-6), 102.17 (C-7), 146.32 (C-8), 108.78 (C-9), 130.5 (C-10), 111.9 (C-11), 19.0 (C-12), 26.0 (C-14), 56.32 (OCH_3), 56.17 (OCH_3).

5,6-Dimethoxy-2-acetylbenzofuran (9). ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm): 6.98 (1H, s, H-3), 7.00 (1H, s, H-4), 6.98 (1H, s, H-7), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 147.57 (C-2), 94.92 (C-3), 113.71 (C-4), 151.29 (C-5), 151.54 (C-6), 102.58 (C-7), 152.19 (C-8), 118.95 (C-9), 187.51 (C-10), 26.03 (C-11), 56.16 (OCH_3), 56.17 (OCH_3).

Palmitoleic Acid-1-monostearate (12). Mp 32–33°C. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm): 4.15 (2H, m, H-1), 3.96 (1H, m, H-2), 3.72 (1H, m, H-3a), 3.66 (1H, m, H-3b), 5.72 (1H, m, H-9), 5.62 (1H, m, H-10). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 174.41, 130.22, 129.68, 70.22, 63.14, 64.35, 34.10, 29.68, 14.32. The TLC and NMR data were identical with those of an author′s sample.

7,8-Dimethoxy-4-methyl-dibenzo-1-carbaldehyde (14). IR (KBr, ν_{max}, cm^{-1}): 1690 (C=O), 1635, 1610, 1570, 1370. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 9.06 (1H, s, 1-CHO), 8.55 (1H, s, H-9), 7.71 (1H, d, J = 8.0, H-2), 7.33 (1H, d, J = 8.0, H-3), 7.15 (1H, s, H-6), 4.08 (3H, s, 8-OMe), 4.01 (3H, s, 7-OMe), 2.66 (3H, s, 4-Me). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 129.02 (C-1), 130.88 (C-2), 125.78 (C-3), 155.48 (C-4a), 152.14 (C-5a), 94.45 (C-6), 151.22 (C-7), 145.76 (C-8), 108.78 (C-9), 115.45 (C-10), 123.12 (C-11), 15.78 (4-CH_3), 56.32 (7-OCH_3), 56.17 (8-OCH_3), 191.20 (CHO).

12,29(30)-Dien-3β-lupeol (16). White powder. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 3.25 (dd, J = 5.2, 12, H-3), 5.50 (t, J = 6, H-12), 4.55 (d, J = 2, H-29α), 4.67 (d, J = 2, H-29β). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 38.62 (C-1), 27.40 (C-2), 79.12 (C-3), 38.80 (C-4), 55.33 (C-5), 18.30 (C-6), 34.20 (C-7), 40.84 (C-8), 50.54 (C-9), 38.76 (C-10),

20.92 (C-11), 122.21 (C-12), 139.86 (C-13), 42.80 (C-14), 37.32 (C-15), 35.38 (C-16), 42.98 (C-17), 48.31 (C-18), 47.70 (C-19), 151.12 (C-20), 26.66 (C-21), 40.12 (C-22), 28.11 (C-23), 15.36 (C-24), 16.21 (C-25), 15.98 (C-26), 14.56 (C-27), 17.83 (C-28), 109.32 (C-29), 19.32 (C-30).

(+)-4-Hydroxypinoresinol-4-*O*-β-D-glucopyranoside (17). White powder. ^{1}H NMR (400 MHz, TMS, acetone-d_6, δ, ppm, J/Hz): 7.18 (1H, d, J = 21.0, H-2′), 6.97 (1H, d, J = 2.0, H-2), 6.79 (1H, d, J = 8.0, H-5), 6.82 (1H, dd, J = 8.0, 2.0, H-6), 4.84 (1H, d, J = 7.6, H-7), 5.69 (2H, br.s, H-9), 6.75 (1H, d, J = 8.0, H-5′), 6.93 (1H, dd, J = 8.0, 2.0, H-6′), 4.94 (1H, d, J = 7.6, H-7′), 4.68 (d, J = 8.0, H-1″), 4.17 (dd, J = 9.2, 5.6, H-9′a), 3.94 (dd, J = 9.2, 2.0, H-9′b), 3.85 (s, OCH_3), 3.82 (s, OCH_3), 3.78 (dd, J = 12.0, 2.0, H-6″a), 3.60 (H-6″b), 3.80 (m, H-2″, 3″, 5″), 3.28 (m, H-8), 2.97 (1H, t, J = 8.0, H-8). ^{13}C NMR (100 MHz, TMS, acetone-d_6, δ, ppm): 133.2 (C-1), 109.2 (C-2), 147.32 (C-3), 145.78 (C-4), 114.0 (C-5), 118.1 (C-6), 82.75 (C-7), 61.0 (C-8), 102.04 (C-9), 133.0 (C-1′), 109.9 (C-2′), 147.12 (C-3′), 145.26 (C-4′), 114.4 (C-5′), 119.4 (C-6′), 88.12 (C-7′), 52.27 (C-8′), 70.7 (C-9′), 97.92 (C-1″), 73.3 (C-2″), 76.4 (C-3″), 70.87 (C-4″), 76.3 (C-5″), 61.53 (C-6″), 55.3 (OCH_3), 55.0 (OCH_3).

β-Sitosterol (19). White needle crystals from Me_2CO, mp 139–140°C. TLC and IR spectrum were identical with those of an author′s sample.

Plant Material. The plant material was collected from the Nanchuan Region of Chongqing, China, in November 2006 and was identified by Dr. Huan-Yang Qi. A voucher specimen (No. 2006L01) has been deposited at the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, P.R. China.

Extraction, Isolation, and Purification of Compounds. Air-dried and ground whole plants of *Ligularia nanchuanica* S. W. Liu (750 g) were extracted four times with 85% EtOH at room temperature, each time lasting three days. The combined extracts were evaporated to dryness under reduced pressure. The residue (70 g) was then suspended in H_2O (0.5 L) and extracted with ethyl acetate (0.5 L × 4) and *n*-butanol (0.5 L × 4) successively. The ethyl acetate extract (38 g) was subjected to column chromatography on silica gel (200–300 mesh, 300 g) using petroleum ether with increasing volume of acetone (from 30: 1 to 1: 1, v/v) as eluent to give nine fractions (Fr. 1–Fr. 9). Fraction 1 (30:1, v/v) was chromatographed on a silica gel column using petroleum ether–ethyl acetate (15:1, v/v) as eluent to yield pure compound **1** (8 mg) and compound **6** (6 mg). Fraction 2 (from 25:1 to 20:1, v/v) was eluted with petroleum ether–acetone (20:1 = v:v) to give Fr. 2a and Fr. 2b. Fraction 2a was repeatedly purified by CC over silica gel with petroleum ether–acetone (20:1, v/v) as eluent to give pure compound **7** (10 mg). Fraction 2b was chromatographed on a silica gel column with petroleum ether–ethyl acetate (12:1, v/v) as eluent to give compound **8** (9 mg). Fraction 3 (from 20:1 to 15:1, v/v) was eluted with petroleum ether–ethyl acetate (15: 1 = v:v) to give five subfractions Fr. 3a–Fr. 3e. Fraction 3a was repeatedly purified by CC over silica gel with petroleum ether–acetone (10:1, v/v) as eluent to give pure compound **4** (10 mg) and compound **5** (35 mg). Fraction 3b was chromatographed on a silica gel column with chloroform–acetone (30:1, v/v) as eluent to give compound **2** (7 mg) and compound **11** (8.5 mg). Fraction 3c was chromatographed on a silica gel column with chloroform–ethyl acetate (20:1, v/v) to give compound **3** (4 mg). Fraction 3d was chromatographed on a silica gel column with chloroform–acetone (15:1, v/v) to give a crude product, then purified by *p*-TLC (petroleum ether–ethyl acetate, 1: 1, v/v) to give compound **9** (10 mg). Colorless needle crystals were obtained from Fr. 3e, compound **19** (20 mg). Fraction 4 (10:1, v/v) was chromatographed on a silica gel column with petroleum ether–acetone (12:1, v/v) to give compound **16** (10 mg) and compound **12** (6.0 mg). Fraction 5 (7:1, v/v) was eluted with petroleum ether–ethyl acetate (7:1 = v:v) to give three subfractions Fr. 5a–Fr. 5c. Fraction 5a was repeatedly purified by CC over silica gel with chloroform–acetone (20:1, v/v) and chloroform–ethyl acetate (4:1, v/v) as eluent to give pure compound **15** (6.4 mg). Fraction 5b was chromatographed on a silica gel column with petroleum ether–acetone (10:1, v/v) as eluent to give compound **14** (10 mg). Fraction 5c was chromatographed on a silica gel column with chloroform–ethyl acetate (10:1, v/v) to give compound **10** (13 mg). Fraction 7 (3:1, v/v) was chromatographed on a silica gel column using petroleum ether–ethyl acetate (3:1, v/v) as eluent to Fr. 7a and Fraction 7b. Fr. 7a was repeatedly purified by CC over silica gel with chloroform–acetone (10:1, v/v) as eluent to give pure compound **13** (10 mg). Fraction 7b was chromatographed on a silica gel column with petroleum ether–acetone (5:1, v/v) as eluent to give compound **17** (10 mg). Compound **18** (28 mg) was crystallized from Fr. 8 (1:1, v/v).

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (NSFC, No. 20372029 and 20475057) and the National Natural Science Foundation of Gansu Province of China (No. 3ZS051-A25-077).

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