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Chemical constituents from the leaves of *Cerbera manghas*

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ABSTRACT

Objective: To study the chemical constituents in leaves of *Cebera manghas*. **Methods**: Chemical constituents were isolated by using various column chromatography and the structures were elucidated on basis of physicochemical constants and spectral data analysis. **Results**: Nine compounds were obtained including p-hydroxybenzaldehyde (1), benzamide (2), n-hexadecane acid monoglyceride(3), loliolide(4), β-sitosterol(5), cerberin(6), neriifolin(7), cerleaside A(8), daucosterol (9). **Conclusions**: Compounds 1-4 are obtained from this genus for the first time.

1. Introduction

Cebera manghas L. is a kind of semi-mangrove plant belonging to Apocynaceae, which is indigenous to tropical and subtropical region. In China, it is widely distributed in Hainan, Guangdong, and Guangxi province. This plant was used by Li nationality as emetic and purgatives medicine in Hainan province[1-2]. Our research group had carried out the chemical constituents of the bark of *Cerbera manghas*, and we discovered several natural products with new and unique structures[3-4]. For further exploitation of this Li nationality medicine, we launch the study of chemical constituents from the leaves.

2. Materials and methods

2.1. General experimental procedures

Melting points were determined on XT-5 melting point apparatus and uncorrected. UV spectra were taken on JASCO-650 spectrophotometer. IR spectra were obtained on a JASCO-FT-IR-4100 infrared spectrophotometer. The NMR data were recorded on Bruker AV 300/600 (300/600MHz for ^{1}H and 75/150MHz for ^{13}C) in d6-DMSO with TMS as internal standard. Chromatography was perform on silica gel column (200-300 mesh, Qingdao Haiyang), Sephadex LH-20 (Pharmacia Biotech Sweden).

2.2. Plant material

The leaves of *Cerbera manghas* were collected from Hainan province, China, in August 2007. A voucher specimen was identified by Prof. Wei-pin Chen and deposited in Hainan Medical College.

2.3. Extraction and isolation

The air-dried leaves were ground into 10 kg of coarse power, which were exhaustively extracted with 70% ethanol. The solvent was removed under reduced pressure to give 1.5 kg of residue. The extract was partitioned with petroleum ether, methylene dichloride, ethyl acetate and n-butanol to afford 120 g of methylene dichloride extract. 100 g of CH_2Cl_2 extract was then subjected to silica gel eluted with petroleum ether-ethyl acetate successively. Seven fractions (Fr.A-G) were collected. 10 mg of β-sitosterol (compound 5) were isolated from Fr.B by recrystallization. 15 mg of p-hydroxybenzaldehyde (compound 1) and 10 mg of benzamide (compound 2) were obtained from Fr.C by using repeated silica gel column chromatography. 20 mg of n-hexadecane acid monoglyceride(compound 3) and 15 mg of loliolide(compound 4) were obtained from

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Fr.D. 50 mg of cerleaside A(compound 8) and 20 mg of daucosterol (compound 9) were obtained from Fr.G. 8 mg of cerberin(compound 6) and 35 mg of neriifolin(compound 7) were isolated from Fr.E and Fr.F separately.

3. Results

3.1. Structures of the isolated compounds

The 70% ethanol extract of the leaves were evaporated *in vacuo*, and then the residue was suspended in water and partitioned with petroleum ether, methylene dichloride, ethyl acetate, and n-butanol. The methylene dichloride extract was separated by repeated column chromatography which led to isolation of the nine compounds (structures see Figure 1).

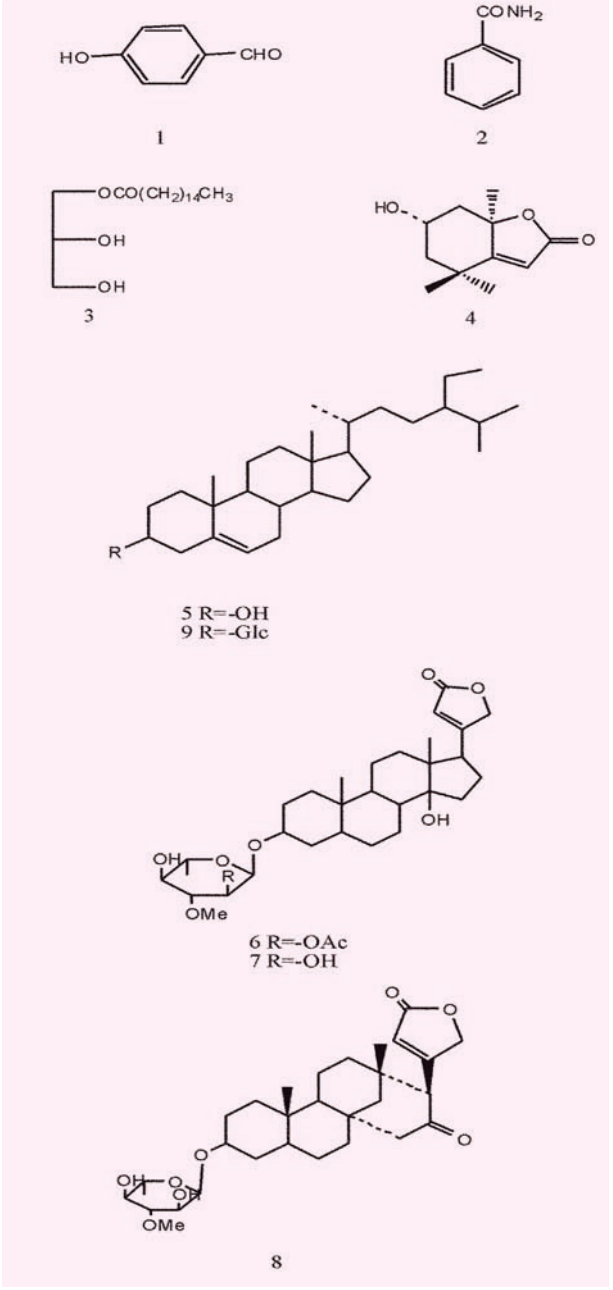

Figure 1. Structures of compounds 1-9.

3.2. Elucidation and spectral data of the compounds

Compound 1: colorless needles, mp 178-182 ℃. ^{1}H-NMR (300 MHz, $CDCl_3$) δ :7.82 (2H, d, *J*=8.4 Hz, H-2,6), 6.97 (2H, d, *J*=8.4 Hz, H-3,4), 9.10 (1H, s, -CHO), 4.96 (1H, brs, -OH).

The above data were identical to *p*-hydroxybenzaldehyde[5], and compound 1 was determined to be *p*-hydroxybenzaldehyde.

Compound 2: colorless needles, mp 180-182 ℃. ^{1}H-NMR (300 MHz, d_6-DMSO) δ :7.89 (2H, m, H-2,6), 7.43 (1H, t, *J*=6.9Hz, H-4), 7.37 (2H, d, *J*=6.9 Hz, H-3,5).

The above data were identical to benzamide[6], and compound 2 was determined to be benzamide.

Compound 3: white powder, mp 64-66 ℃. IR (KBr) υ cm^{-1}: 3 436, 2 955, 2 916, 2 848, 1 705, 1 473, 1 464, 1 435, 1298, 935, 729, 719, 688. ^{1}H-NMR (300 MHz, $CDCl_3$) δ : 4.20 (1H, dd, *J*=12.3, 6.0 Hz, H-1a), 4.14 (1H, dd, *J*=12.3, 6.3 Hz, H-1b), 3.95 (1H, m, H-2′), 3.72 (1H, dd, *J*=14.2, 6.0 Hz, H-3′a), 3.60 (1H, dd, *J*=14.2, 3.0 Hz, H-3′b), 2.36 (2H, t, *J*=7.3 Hz, H-2), 1.63 (2H, m, H-3), 1.25 (28H, brs, $-CH_2$), 0.88 (3H, t, *J*=6.4 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ : 65.1 (C-1′), 70.2 (C-2′), 63.3 (C-3′), 174.4 (C-1), 34.1 (C-2), 31.9 (C-14), 24.9 (C-3), 22.7 (C-15), 14.1 (C-16), 29.1-29.7 (C-4-13).

The above data were identical to n-hexadecane acid monoglyceride[7], and compound 3 was determined to be n-hexadecane acid monoglyceride.

Compound 4: colorless needles, mp 139-140 ℃. ^{1}H-NMR(300 MHz, d_6-DMSO) δ : 5.78 (1H, s, H-7), 4.98 (1H, d, *J*=3.3 Hz, -OH), 4.10 (1H, brs, H-3), 2.28 (1H, dd, *J*=13.2, 4.8Hz, H-4 α), 1.87 (1H, dd, *J*=13.2, 2.1Hz, H-2 α), 1.59 (1H, m, H-4 β), 1.42 (1H, m, H-2 β), 1.59, 1.36, 1.18 (each 3H, s, 3×CH_3). ^{13}C-NMR (75 MHz, d_6-DMSO) δ : 183.1 (C-6), 171.0 (C-8), 112.1 (C-7), 86.5 (C-5), 64.1 (C-3), 46.7 (C-4), 45.3 (C-2), 35.7 (C-1), 30.6 (C-9), 26.8 (C-11), 26.2 (C-10).

The above data were identical to loliolide[8], and compound 4 was determined to be loliolide.

Compound 5: white needles, mp 135-137 ℃. IR (KBr) υ cm^{-1}: 3 424 (OH), 2 958, 2 869 (CH2), 1 637 (C=C), 1 466, 1 040 (C-O). ^{1}H-NMR (600 MHz, CDCl3) δ : 5.36 (1H, d, *J*=2.4 Hz, H-6), 3.55 (1H, m, H-3), 1.03 (3H, s, -Me), 0.94 (3H, d, *J*=6.0 Hz, -Me), 09.0-0.80 (9H, m, -Me), 0.70 (3H, s, -Me). ^{13}C-NMR (125 MHz, $CDCl_3$) δ :37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.1 (C-17), 12.0 (C-18), 19.4 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.0 (C-28), 11.8 (C-29).

The above data were identical to β-sitosterol[9], and compound 5 was determined to be β-sitosterol.

Compound 6: colorless needles, mp 210-215 ℃. IR (KBr) υ cm^{-1}: 3 460, 2 921, 1 745, 1 715, 1 460, 1 358, 1 060. ^{1}H-NMR (300 MHz, CD_3COCD_3) δ : 0.92, 0.96 (3H each, s, H-18, 19), 1.17 (3H, d, *J*=6.6 Hz, 6′-CH_3), 2.76 (3H, s, 2′-OAc), 2.84 (1H, m, H-16), 3.55 (3H, s, 3′-OCH_3), 4.76 (1H, d, *J*=3.6 Hz, H-1′), 3.20-3.70 (4H, m), 4.07 (1H, d, *J*=4.5 Hz, H-2′), 3.18 (1H, s, 14-OH), 4.87 (2H, H-21), 5.86 (1H, s, H-22). ^{13}C-NMR (75 MHz, d_6-DMSO δ :176.2 (C-23), 174.3 (C-20), 117.7 (C-22), 98.5 (C-1′), 85.5 (C-14), 85.3 (C-3′), 76.5

(C-4′), 73.9 (C-3, 21), 73.6 (C-2′), 68.6 (C-5′), 60.6 (-OMe), 51.7 (C-17), 50.5 (C-13), 42.5 (C-8), 40.4 (C-7), 37.4 (C-5), 36.3 (C-9), 36.0 (C-10), 35.9 (C-12), 33.5 (C-15), 31.3 (C-4), 30.5 (C-1), 27.6 (C-2, 6), 27.2 (C-16), 24.2 (C-19), 22.1 (C-7), 22.0 (C-11), 18.6 (C-18, 6′).

The above data were identical to cerberin[10], and compound 6 was determined to be cerberin.

Compound 7: colorless needles, mp 239-241 ℃. ^{1}H-NMR (300 MHz, d_6-DMSO) δ: 5.91 (1H, s, H-22), 5.03 (1H, d, *J*=6.0 Hz, H-1′), 4.85 (2H, H-21), 4.63 (1H, d, *J*=3.9 Hz, 2′-OH), 4.55 (1H, d, *J*=7.2 Hz, H-2′), 3.52 (1H, brs, 14-OH), 3.10-3.75 (5H, m), 3.48 (3H, 3′-OMe), 2.84 (1H, m, H-16), 1.07 (3H, d, *J*=6.6 Hz, 6′-Me), 0.91, 0.82 (3H each, s, H-18,19).

The above data were identical to neriifolin[11], and compound 7 was determined to be neriifolin.

Compound 8: colorless needles. ^{1}H-NMR (300 MHz, d_6-DMSO) δ: 5.54 (1H, s, H-22), 5.04 (1H, d, *J*=6.0Hz, H-1′), 4.74 (2H, d, *J*=18.0 Hz, H-21), 4.60 (1H, d, *J*=3.6 Hz, -OH), 4.53 (1H, d, *J*=7.2 Hz, H-2′), 4.09 (1H, s, -OH), 3.76 (1H, brs, H-3), 3.51 (1H, m, H-5′), 3.50 (3H, 3′-OMe), 3.00-3.23 (3H, m, H-17, 3′, 4′), 2.82 (1H, m, H-16a), 1.06 (3H, d, *J*=6.6 Hz, 6′-Me), 0.80, 0.69 (3H each, s, H-18,19). ^{13}C-NMR (75 MHz, d_6-DMSO) δ: 221.7 (C-14), 173.5 (C-23), 172.8 (C-20), 114.6 (C-22), 97.2 (C-1′), 83.2 (C-3′), 75.3 (C-4′), 73.0 (C-2′), 72.2 (C-21), 71.8 (C-3), 67.6 (C-5′), 59.8 (-OMe), 51.8 (C-17), 48.3 (C-8), 47.0 (C-13), 45.0 (C-9), 43.3 (C-15), 42.0 (C-12), 37.0 (C-10), 36.3 (C-5), 31.4 (C-1), 29.5 (C-2), 26.3 (C-4), 28.6 (C-6), 26.0 (C-16), 25.9 (C-19), 23.9 (C-7), 23.0 (C-18), 20.7 (C-11), 17.8 (C-6′).

The above data were identical to cerleaside A[12], and compound 8 was determined to be cerleaside A.

Compound 9: white powder, mp 289-292 ℃. IR (KBr) υ cm^{-1}: 3 420, 2 931, 2 869, 2 848, 1 646, 1 540, 1 456, 1023, 910. ^{1}H-NMR (300 MHz, C5D5N) δ: 5.37 (1H, brs, H-6), 5.20 (1H, d, *J*=7.8 Hz, H-1′), 4.01-4.61 (6H, m), 1.00 (3H, d, *J*=6.6 Hz, $-CH_3$), 0.95 (3H, s, $-CH_3$), 0.94 (3H, d, *J*=6.6 Hz, $-CH_3$), 0.90 (3H, d, *J*=6.6 Hz, $-CH_3$), 0.88 (3H, t, $-CH_3$), 0.67 (3H, s, $-CH_3$).

The above data were identical to daucosterol[13], and compound 9 was determined to be daucosterol.

4. Discussion

It is reported that the seed of *Cerbera manghas* is extremely poisonous. Feeding one seed by accident can cause death. Cerberin is a sort of cardenolides, which is the toxic component of the seeds. In our study, we found that the content of cerberin in the leaves is high. The conclusion can be drawn that the leaves are also poisonous, and it is hazardous if ingested by animals or human.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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