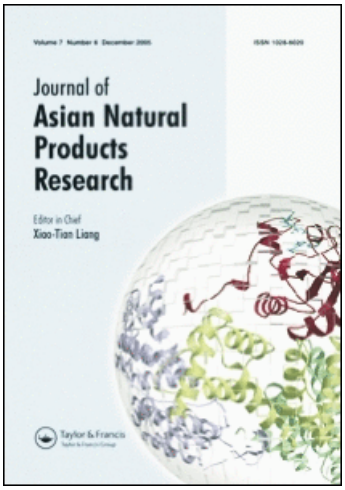

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Chemical study on *Cyathula officinalis* Kuan

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Four new compounds, 4-[(1-ethoxy-2-hydroxy)ethyl]phenol (**1**), 2,3-isopropylidene cyasterone (**2**), 24-hydroxycyasterone (**3**) and 2,3-isopropylidene isocyasterone (**4**), together with fourteen known compounds, have been isolated from the roots of *Cyathula officinalis* Kuan. Their structures have been elucidated predominantly by spectroscopic methods.

Keywords: *Cyathula officinalis* Kuan; Phytoecdysterone; Cyasterone; Isocyasterone; Phenol

1. Introduction

The air-dried roots of *Cyathula officinalis* Kuan, a famous traditional Chinese medicinal herb, possess functions of removing blood stasis and restoring menstrual flow, easing joint movement, and inducing diuresis for treating stranguria [1]. Previous studies on the roots of *C. officinalis* Kuan led to the isolation of phytoecdysteroids [2–6], polysaccharide [7] and isoflavones [8]. To establish HPLC fingerprint analysis, a systematic chemical study on the roots of *C. officinalis* Kuan has been carried out. From the ethanolic extract 18 compounds were isolated. On the basis of spectral data, they were determined to be 4-[(1-ethoxy-2-hydroxy)ethyl]phenol (**1**), 2,3-isopropylidene cyasterone (**2**), 24-hydroxycyasterone (**3**), 2,3-isopropylidene isocyasterone (**4**), *N*-[(3,4,5-trimethoxy)phenyl]formamide (**5**) [9], 6,7-dimethoxycoumarin (**6**) [10], butanedioic acid (**7**), *p*-hydroxybenzoic acid (**8**) [11], betavulgarin (**9**) [12,13], daidzin (**10**) [14], puerarin (**11**) [14,15], phenyl 1-β-D-glucopyranoside (**12**) [16], cyasterone (**13**) [17], sengosterone (**14**) [18], amarasterone A (**15**) [3], precyasterone (**16**) [5], isocyasterone (**17**) [4] and makisterone B (**18**) [19]. Compounds **1**–**4** (figure 1) are new. Compounds **5**, **7**, **8**, **10**–**12** and **18** were isolated from the roots of *C. officinalis* Kuan for the first time.

2. Results and discussion

Compound **1** was obtained as colorless crystals. When treated with 3% $FeCl_3$ (aq.), its acetone solution turned blue. The molecular formula was determined as $C_{10}H_{14}O_3$ by

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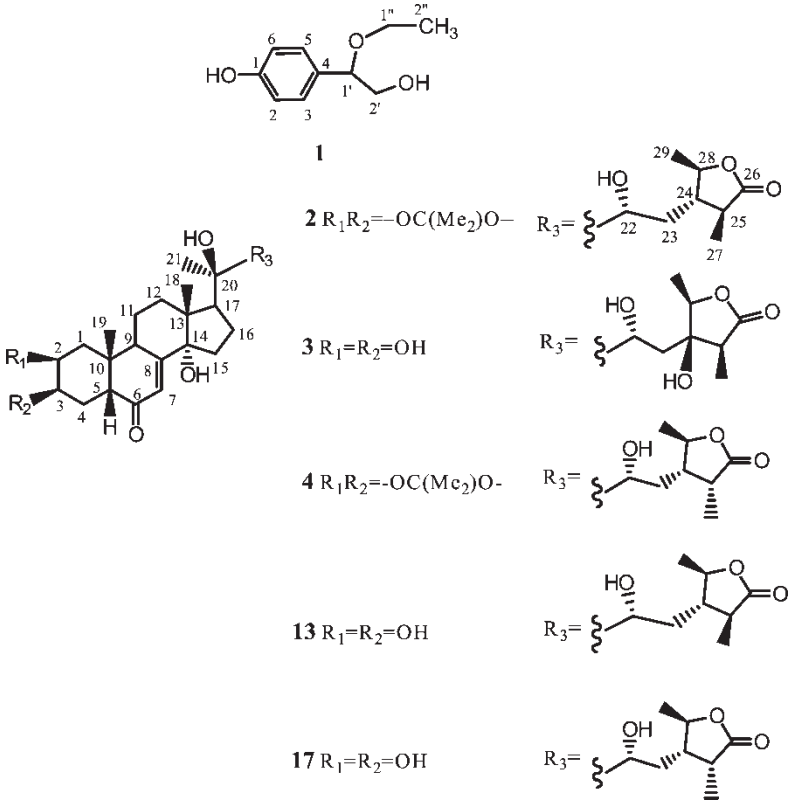


Figure 1. Structures of compounds **1**–**4**, **13**, and **17**.

the quasi-molecular peak at *m/z* 181.0857 $[M - H]^-$ in negative ion HR-ESIMS spectrum. Its IR spectrum shows absorption bands for hydroxyl (3400 cm^{-1}) and aromatic ring (1600, 1510, 1465 cm^{-1}). The ^1H NMR signals at δ 7.15 and 6.81 (each 2H, d, $J = 8.4$ Hz) indicate a 1,4-disubstituted phenyl moiety. The signal at δ 8.26 (1H, s) is derived from phenolic hydroxyl proton. The presence of an ethoxyl group is concluded from the ^1H NMR signals at δ 3.37 (2H, m) and 1.15 (3H, t, $J = 7.2$ Hz), and ^{13}C NMR signals at δ 63.7 and 14.8. ^{13}C NMR signals at δ 82.8 and 67.0 suggest two oxygenated C-atoms. Accordingly, **1** could be determined as either 4-[(1-ethoxy-2-hydroxy)ethyl]phenol or 4-[(2-ethoxy-1-hydroxy)ethyl] phenol. From the HMBC experiment (figure 2) the structure of **1** was determined as 4-[(1-ethoxy-2-hydroxy)ethyl]phenol.

Compound **2** was obtained as colorless needles. Its molecular formula was determined as $C_{32}H_{48}O_8$ by the quasi-molecular peak at *m/z* 559.3251 $[M - H]^-$ in its negative ion HR-ESIMS spectrum. Its IR spectrum shows absorption bands for hydroxyl (3456 cm^{-1}), γ-lactone (1752 cm^{-1}) and α,β-unsaturated ketone (1643 cm^{-1}). The Liebermann-Burchard reaction of **2** gave a purple visualization. The ^1H NMR signals for seven methyl groups appear at δ 1.45 (3H, s, H-21), 1.38 (3H, d, $J = 6.4$ Hz, H-29), 1.26 (3H, d, $J = 6.8$ Hz, H-27), 1.31, 1.27 (each 3H, s, protons of -Me), 1.00 (3H, s, H-18), and 0.98 (3H, s, H-19). These data suggest that **2** might be an ecdysterone. Comparison of the ^1H NMR spectrum of **2** with that of cyasterone (**13**) shows that compound **2** has two more methyl groups, at δ 1.31 (3H, s) and 1.27 (3H, s). The ^{13}C NMR spectrum of **2** contains three signals more, at δ 108.4 (O–C–O),

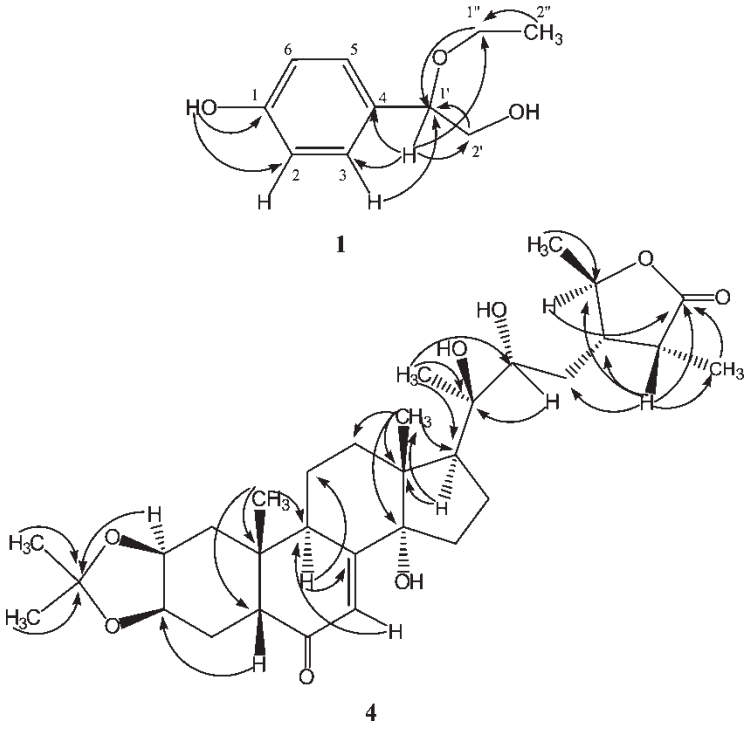
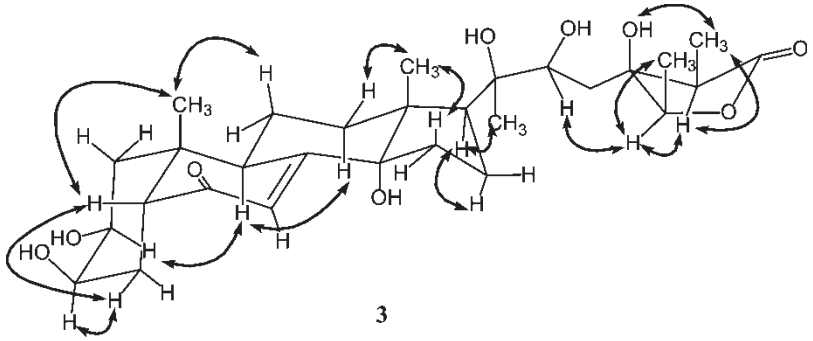


Figure 2. Key HMBC correlations of **1** and **4**.

28.5 (-Me) and 26.4 (-Me), than that of **13**. The signals for C-2, C-3 and C-4 shifted from δ 67.8, 68.0 and 31.7 in **13** to 72.1, 71.6 and 26.7 in **2** respectively. Acid hydrolysis of **2** with 3 M HCl (aq.) afforded **13**. The above evidence indicates that **2** is 2,3-isopropylidene cyasterone.

Compound **3** was obtained as white powder. It turned purple upon reacting with the Liebermann–Burchard reagent. Hydroxyl (3436 cm^{-1}), γ-lactone (1756 cm^{-1}) and α,β − unsaturated carbonyl (1646 cm^{-1}) groups are recognized from its IR spectrum. In the ^{1}H NMR spectrum, five methyl groups resonate at δ 1.61 (3H, s, H-21), 1.58 (3H, d, $J = 6.6$ Hz, H-29), 1.57 (1H, d, $J = 7.2$ Hz, H-27), 1.25 (3H, s, H-18) and 1.08 (3H, s, H-19). Thus, **3** could be an ecdysterone. From the ^{13}C NMR spectra of **3** and cyasterone (**13**), it is concluded that compound **3** has an oxygenated quaternary carbon at δ 78.9 instead of the C-24 methine in cyasterone (**13**). The multiplicity of the ^{1}H NMR signals of **3** at δ 4.59 (1H, q, $J = 6.6$ Hz, H-28) and 3.14 (1H, q, $J = 7.2$ Hz, H-25) as well as the molecular formula $C_{29}H_{44}O_9$ provided by the ion peak at 559.2884 $[M + Na]^+$ in the HR-ESIMS spectrum confirm that **3** is 24-hydroxycyasterone. Comparison of ^{13}C NMR signals for C-20–C-22 (δ 76.4, s; 20.7, q; 73.3, d; respectively) in **3** with those of 20,22-dihydroxy sterols with different configurations (20α,22β: δ 77.5, 23.8, 76.2; 20β,22α: δ 76.5, 21.1, 76.5; 20α,22α: δ 77.3, 24.2, 77.9; 20β,22β: δ 76.5, 21.9, 78.3) suggest the presence of 20β and 22α hydroxyl groups in **3** [20]. The relative stereochemistry of **3** was assigned by a NOESY experiment (figure 3).

Compound **4** was obtained as colorless needles. Its molecular formula was determined as $C_{32}H_{48}O_8$ by the ion peak at *m/z* 559.3271 $[M - H]^-$ in its negative ion HR-ESIMS spectrum. The IR spectrum shows absorption bands for hydroxyl (3458 cm^{-1}), γ-lactone



3

Figure 3. Key NOESY of correlations of **3**.

(1754 cm^{-1}) and α,β-unsaturated ketone (1656 cm^{-1}). The Liebermann–Burchard reaction of **4** gave a purple visualization. In the ^{1}H NMR spectrum, seven methyl signals appear at δ 1.59 (3H, s, H-21), 1.26 (3H, d, $J = 6.6$ Hz, H-29), 1.25 (3H, d, $J = 7.8$ Hz, H-27), 1.54, 1.30 (each 3H, s, protons of -Me), 1.18 (3H, s, H-18), and 0.99 (3H, s, H-19). Thus, **4** may be an ecdysterone. Comparing the ^{1}H NMR spectrum of **4** with that of **17**, compound **4** has two other methyl signals, at δ 1.54 (3H, s) and 1.30 (3H, s). Three more signals at δ 107.9 (O–C–O), 28.6 (-Me) and 26.4 (-Me) were recognized from the ^{13}C NMR spectrum of **4** than for **17**. The signals for C-2, C-3 and C-4 shift from δ 68.1, 68.0 and 29.9 in **17** to 72.2, 71.9 and 26.7 in **4**, respectively. Acid hydrolysis of **4** with 3 M HCl (aq.) yielded **17**. Thus, compound **4** was determined as 2,3-isopropylidene isocyasterone. The HMBC experiment (figure 2) confirmed this conclusion.

Compounds **2** and **4** are derivatives of **13** and **17**, respectively**.** To investigate whether compound **2** is an artifact, compound **13** was added to a mixture of acetone–light petroleum (4:1, v/v) and silica gel (1.5 g) according to the chromatographic conditions for isolating **2**. After stirring the mixture at room temperature (10°C) for two days, no reaction had taken place; in addition, no reaction took place at 30, 50 and then 70°C for two days. Both 20- and 22-OH of cyasterone have been reported to be involved in the first acetonide formation in the presence of an acid like *p*-toluenesulfonic acid at room temperature [21]. Consequently, **2** and **4** may be not an artifact. This conclusion was further confirmed by the detection by HPLC analysis of **2** and **4** in the EtOAc fraction of the EtOH extract.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XRC-1 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 341 automatic polarimeter. UV spectra and IR spectra were recorded on a Lambda 35 spectrometer and a Perkin-Elmer spectrum one FT-IR spectrometer (KBr disc), respectively. Mass spectra were obtained on a Finnigan-LCQ^{DECA} mass spectrometer (ESIMS), an API Q-STAR PULSAR i mass spectrometer (HR-ESIMS) and a VG7070E mass spectrometer (EIMS). NMR spectra were recorded on a Bruker Advance 600 spectrometer with TMS as internal standard. GC was carried out on a Varian CP-3380 gas chromatograph with a chirasil DEX CB column (ϕ 0.25 mm × L 25 m). HPLC was carried out

on a Perkin-Elmer Series 200 with Merck KGaA-Diol (ϕ 4 mm × *L* 250 mm, 5 μm) column. Silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd.) and Al_2O_3 (neutral, 200–300 mesh, Shanghai Ludu Chemical Reagent Plant) were used for column chromatography. TLC was carried out with precoated silica gel GF_{254} plates and visualized by spraying with 7% H_2SO_4 (95% EtOH) followed by heating or under UV light (254 and 365 nm). All solvents used were distilled before use. Solvent systems for TLC: (A) $CHCl_3$–CH_3OH 20:1 (v/v); (B) n-hexane–acetone 3:1 (v/v); (C) light petroleum (bp 60–90°C)–ethyl acetate 1.5:1 (v/v).

3.2 Plant material

Roots of *Cyathula officinalis* Kuan were collected in November 2000 from Jin Kou He district of Leshan, China. The plant sample was identified by Prof. Fa-Ding Fu at Chengdu Institute of Biology, the Chinese Academy of Sciences (CAS). A voucher specimen (A-160) is deposited in the Department of Natural Products Research, Chengdu Institute of Biology, CAS.

3.3 Extraction and isolation

Air-dried, powdered roots of *C. officinalis* Kuan (8 kg) were percolated (25 L × 3) with 95% ethanol at room temperature. After evaporated to dryness under reduced pressure a residue was obtained (801 g). The residue was suspended in water (1000 ml) and extracted with light petroleum (60–90°C, 2.5 L × 7), EtOAc (2.5 L × 10) and n-BuOH (2.5 L × 8) to give the corresponding fractions A (52.5 g), B (32 g) and C (112 g). Fraction A was chromatographed over silica gel (1000 g, ϕ 9 × *L* 50 cm) eluted with light petroleum (60–90°C)–acetone (100:1–1:2, v/v) to give subfractions A_1–A_{15} on the basis of TLC analysis. Subsequent purification of A_8 (2.5 g) by column chromatography over silica gel (ϕ 3.5 × *L* 28 cm) eluted with light petroleum (60–90°C)–acetone (10:1, v/v) afforded **5** (15 mg) and **9** (5 mg). Fraction B was subjected to column chromatography (800 g, ϕ 8 × *L* 50 cm) over silica gel eluted with $CHCl_3$–MeOH (90:1–0:1, v/v) to give subfractions B_1–B_{12} based on TLC analysis. The separation of B_2 (1.6 g) by column chromatography (ϕ 3 × *L* 28 cm) over silica gel eluted with $CHCl_3$–MeOH (30:1, v/v) afforded **6** (89 mg), **2** (12 mg) and **4** (6 mg). B_3 (1.2 g) was separated by column chromatography (ϕ 2 × *L* 25 cm) over silica gel eluted with $CHCl_3$–MeOH (18:1, v/v) to give **1** (15 mg) and **12** (8 mg). By silica-gel column chromatography (ϕ 2 × *L* 20 cm) eluted with $CHCl_3$–MeOH (15:1, v/v), **14** (65 mg) was isolated from B_4 (0.9 g). Compound **13** (3.2 g) was obtained from B_5 (8 g) by column chromatography (ϕ 7.5 × *L* 50 cm) over silica gel eluted with $CHCl_3$–MeOH (13:1, v/v). The separation of B_8 (3.4 g) by column chromatography over Al_2O_3 (200–300 mesh, ϕ 5 × *L* 38 cm) eluted with EtOAc–MeOH (10:1, v/v) yielded **3** (14 mg), **16** (28 mg), **18** (6 mg), **15** (18 mg) and **17** (9 mg). By silica-gel column chromatography (ϕ 6 × *L* 37 cm) eluted with $CHCl_3$–MeOH (8:1, v/v), **10** (35 mg) and **11** (31 mg) were obtained from B_{10} (3.7 g). Compounds **7** (17 mg) and **8** (13 mg) were isolated from fraction B_{11} (1.7 g) by column chromatography over silica gel (ϕ 3.5 × *L* 27 cm) eluted with $CHCl_3$–MeOH (5:1, v/v).

3.3.1 Acidic hydrolysis of 2 and 4. A mixture of **2** (2 mg) and 3 M HCl (aq.) (1 ml) was allowed to stand at room temperature for 10 min to give cyasterone, which was identified by comparison with authentic samples on TLC with solvent systems A, B, and C. Similarly, **4** (2 mg) was added to 3 M HCl (aq.) (1 ml) at room temperature. After 10 min, isocyasterone was detected along with authentic sample on TLC with solvent systems A, B, and C.

3.3.2 Reaction of cyasterone in acetone. Cyasterone (**13**, 10 mg) was added to a mixture of acetone–light petroleum (60–90°C) (4:1) and silica gel (1.5 g). After stirring for two days at 10, 30, 50 and 70°C no reaction had taken place based on TLC analysis.

3.3.3 Analysis of 2 and 4 by HPLC. The ethanolic extract of the roots of *C. officinalis* Kuan was fractionated with light petroleum (60–90°C), EtOAc and n-BuOH. The retention times of compounds **2** and **4** in the EtOAc fraction on HPLC were 20.59 and 21.99 min, respectively. Conditions for HPLC: solvents, CH_2Cl_2–CH_3OH (98:2, v/v); flow rate, 1 ml min^{-1}; column pressure, 1020 psi; detection wavelength, 243 nm.

3.3.4 Analysis of 1 by GC. The retention times of compound **1** were 16.332 min (A, 97.06%) and 19.698 min (A, 0.59%) under the following conditions: column temperature 160°C; eluent gas: N_2; column inlet pressure (9 psi). Thus, compound **1** is an optically pure compound.

3.3.5 4-[(1-Ethoxy-2-hydroxy)ethyl]phenol (1). Colorless crystals (acetone), mp 99–100°C; $[\alpha]_D^{25} + 2.0°$ (*c* 0.1, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3400, 1600, 1510, 1465. ESIMS *m/z* 181 $[M - H]^-$ (negative mode), 205 $[M + Na]^+$ (positive mode). HR-ESIMS (negative mode) *m/z* 181.0857 ($[M - 1]^-$, calcd for $C_{10}H_{13}O_3$, 181.0864). ^{1}H NMR (600 MHz, acetone-d_6) δ (ppm): 8.26 (1H, s, 1-OH), 7.15 (2H, d, $J = 8.4$ *Hz*, H-3, 5), 6.81 (2H, d, $J = 8.4$ Hz, H-2, 4), 4.27 (1H, dd, $J = 7.8, 4.2$ Hz, H-1′), 3.57 (1H, m, H-2′), 3.45 (1H, m, H-2′), 3.37 (2H, m, H-1″), 1.15 (3H, t, $J = 7.2$ Hz, H-2″). ^{13}C NMR (150 MHz, acetone-d_6) δ (ppm): 156.9 (C-1), 131.0 (C-4), 128.0 (C-3, 5), 115.0 (C-2, 6), 82.8 (C-1′), 67.0 (C-2′), 63.7 (C-1″), 14.8 (C-2″).

3.3.6 2,3-Isopropylidene cyasterone (2). Colorless needles ($CHCl_3$–MeOH), mp 241–243°C; $[\alpha]_D^{25} + 30.9°$ (*c* 0.11, MeOH). UV λ_{max} (nm) (log ε) in MeOH: 243 (3.95). IR (KBr) ν_{max} (cm^{-1}): 3456, 1752, 1643; ESIMS *m/z* 559 $[M - H]^-$ (negative mode). 583 $[M + Na]^+$, 1143 $[2M + Na]^+$ (positive modes); HR-ESIMS (negative mode) *m/z* 559.3251 ($[M - 1]^-$, calcd for $C_{32}H_{47}O_8$, 559.3270). ^{1}H NMR (400 MHz, acetone-d_6 and C_5D_5N) δ (ppm): 5.84 (1H, d, $J = 2.0$ Hz, H-7), 4.18 (1H, br.s, H-3), 4.16 (1H, m, H-2), 4.10 (1H, dq, $J = 9.6$ Hz, 6.4 Hz, H-28), 3.70 (1H, d, $J = 10.0$ Hz, H-22), 3.03 (1H, t, $J = 8.4$ Hz, H-9), 2.56 (1H, t, $J = 9.2$ Hz, H-17), 2.42 (1H, dq, $J = 10.8$ Hz, 6.8 Hz, H-25), 2.27 (1H, d, $J = 11.6$ Hz, H-5), 2.18 (1H, q, $J = 10.8$ Hz, H-16a), 1.45 (3H, s, H-21), 1.38 (3H, d, $J = 6.4$ Hz, H-29), 1.26 (3H, d, $J = 6.8$ Hz, H-27), 1.31, 1.27 (each 3H, s, protons of isopropylidene), 1.00 (3H, s, H-18), 0.98 (3H, s, H-19). ^{13}C NMR data: see table 1.

3.3.7 24-Hydroxycyasterone (3). White powder, mp 181–183°C; $[\alpha]_D^{25} + 36.3°$ (*c* 0.16, MeOH). UV λ_{max} (nm) (log ε) in MeOH: 243 (3.99). IR ν_{max} (KBr) (cm^{-1}): 3436, 1755.9, 1646. ESIMS *m/z* 535 $[M - H]^-$, 1071 $[2M - H]^-$ (negative modes). 559 $[M + Na]^+$, 1095 $[2M + Na]^+$ (positive modes). HR-ESIMS (positive mode) *m/z* 559.2884 ($[M + Na]^+$, calcd for $C_{29}H_{44}O_9Na$, 559.2883). EI-MS (70 eV) *m/z* (rel. int.%) 500 $[M - 2H_2O]$, 482 $[M - 3H_2O]$, 363 (3), 345 (8), 327 (12), 301 (7), 105 (9), 91 (12), 83 (9), 81 (10), 79 (8), 55 (23), 43 (100), 40 (61), 29 (49); ^{1}H NMR (600 MHz, C_5D_5N,) δ (ppm): 6.30 (1H, d, $J = 2.4$ Hz, H-7), 4.59 (1H, q, $J = 6.6$ Hz, H-28), 4.28 (1H, d, $J = 10.8$ Hz, H-22), 4.26 (1H, br.s, H-3), 4.20 (1H, m, H-2), 3.60 (H, br.s, H-9), 3.14 (1H, q, $J = 7.2$ Hz, H-25), 3.04 (1H, dd, $J = 3.6, 13.2$ Hz, H-5), 2.90 (1H, t, $J = 9.0$ Hz, H-17), 2.63 (1H, dt, $J = 12.6, 4.2$ Hz, H-12e), 2.54 (1H, q, $J = 10.2$ Hz, H-16a), 1.61 (3H, s, H-21), 1.58 (3H, d, $J = 6.6$ Hz, H-29),

Table 1. ^{13}C NMR data of **2**–**4** (δ in ppm).

C	***2***[a]	***2***[b]	***3***[c,d]	***4***[c,d]
1	37.6	37.9	38.4 t	38.0 t
2	72.1	72.5	67.8 d	72.2 d
3	71.6	72.1	67.8 d	71.9 d
4	26.7	26.8	32.1 t	26.7 t
5	50.8	51.5	51.5 d	51.3 d
6	202.6	201.7	203.2 s	202.0 s
7	121.7	121.2	121.6 d	121.8 d
8	162.6	164.4	165.5 s	165.1 s
9	34.4	34.9	34.4 d	34.8 d
10	37.8	38.2	37.2 s	37.8 s
11	20.5	21.0	20.8 t	20.9 t
12	31.3	31.3	31.8 t	31.4 t
13	48.2	49.0	48.0 s	48.1 s
14	84.5	84.1	83.9 s	83.8 s
15	31.7	31.9	31.6 t	31.7 t
16	20.3	20.5	21.2 t	21.4 t
17	48.9	49.7	49.7 d	49.7 d
18	17.4	17.5	17.6 q	17.5 q
19	23.6	23.6	24.2 q	23.6 q
20	77.0	76.6	76.4 s	76.5 s
21	20.4	21.0	20.7 q	21.1 q
22	73.2	74.0	73.3 d	74.8 d
23	33.7	34.1	37.8 t	26.4 t
24	47.7	48.2	78.9 s	39.6 d
25	42.3	42.3	45.6 d	26.4 d
26	179.1	178.6	178.3 s	179.8 s
27	15.7	15.4	8.9 q	12.6 q
28	80.1	79.8	82.1 d	78.4 d
29	19.6	19.2	12.8 q	17.3 q
OCO	108.4	108.1		107.9 s
Me	28.5	28.5		28.6 q
	26.4	26.3		26.4 q

[a]In $CDCl_3$ at 150 MHz. [b]In acetone-d_6 and C_5D_5N at 100 MHz. [c]Multiplicity determined by DEPT. [d]In C_5D_5N at 150 MHz.

1.57 (1H, d, $J = 7.2$ Hz, H-27), 1.25 (3H, s, H-18), 1.08 (3H, s, H-19). ^{13}C NMR data: see table 1.

3.3.8 2,3-Isopropylidene isocyasterone (4). Colorless needles ($CHCl_3$–MeOH); mp 260–262°C. $[\alpha]_D^{25} + 42.2°$ (*c* 0.12, MeOH). UV λ_{max} (nm) (log ε) in MeOH: 242 (3.98). IR ν_{max} (KBr) (cm^{-1}): 3458, 1754, 1656. ESIMS *m/z* 559 $[M - H]^-$ (negative mode), 583 $[M + Na]^+$ and 1143 $[2M + Na]^+$ (positive modes). HR-ESIMS (negative mode) *m/z*: 559.3271 ($[M - 1]^-$, calcd for $C_{32}H_{47}O_8$, 559.3270). ^{1}H NMR (600 MHz, C_5D_5N) δ (ppm): 6.19 (1H, s, H-7), 4.63 (1H, qui, $J = 6.6$ Hz, H-28), 4.18 (1H, m, H-2), 4.08 (1H, br.s, H-3), 3.80 (1H, d, $J = 10.8$ Hz, H-22), 3.58 (1H, br.s, H-9), 3.19 (1H, qui, $J = 7.8$ Hz, H-25), 3.13 (1H, t, $J = 9.0$ Hz, H-17), 2.56 (1H, d, $J = 12.0$ Hz, H-5), 2.48 (1H, q, $J = 11.2$ Hz, H-16), 1.59 (3H, s, H-21), 1.54, 1.30 (each 3H, s, protons of isopropylidene), 1.26 (3H, d, $J = 6.6$ Hz, H-29), 1.25 (3H, d, $J = 7.8$ Hz, H-27), 1.18 (3H, s, H-18), 0.99 (3H, s, H-19). ^{13}C NMR data: see table 1.

References

[1] National Pharmacopoeia Committee, *Pharmacopoeia of People's Republic of China*, Chemical Industry Press, Beijing (2000), p. 28.
[2] H. Hikino, Y. Hikino, T. Nomoto, T. Takemoto. *Tetrahedron Lett.*, **9**, 4929–4932 (1968).

[3] T. Takemoto, K. Nomoto, H. Hikino. *Tetrahedron Lett.*, **9**, 4953–4956 (1968).
[4] H. Hikino, K. Nomoto, T. Takemoto. *Chem. Pharm. Bull.*, **19**, 433–435 (1971).
[5] H. Hikino, K. Nomoto, R. Ino, T. Takemoto. *Chem. Pharm. Bull.*, **18**, 1078–1081 (1970).
[6] H. Hikino, K. Nomoto, T. Takemoto. *Tetrahedron Lett.*, **11**, 887–898 (1970).
[7] X.M. Chen, G.Y. Tian. *Chin. J. Chem.*, **21**, 858–863 (2003).
[8] Q.Y. Cheng, X.C. Zhan, L.G. He, Y.D. Zhang. *J. Chin. Med.*, **12**, 203–210 (2001).
[9] B. Qadri, S. Harkar, E.S. Waight. *J. Heterocycl. Chem.*, **26**, 957–964 (1989).
[10] R.T. Laureola. *Phytochemistry*, **26**, 2063–2069 (1987).
[11] M.F. Wang, H. Kikuzaki, N.Q. Zhu, S.M. Sang, N. Nakatani, C.T. Ho. *J. Agric. Food Chem.*, **48**, 235–238 (2000).
[12] T.T. Jong, C.C. Hwang. *Planta Med.*, **61**, 584–585 (1995).
[13] C.A. Elliger, J.M. Halloin. *Phytochemistry*, **37**, 691–693 (1994).
[14] Y. Ohshima, T. Okuyama, K. Takahashi, T. Takizawa, T. Shibata. *Planta Med.*, **54**, 250–254 (1988).
[15] G. Cheng, Y.J. Bai, Y.Y. Zhao, J. Tao, Y. Liu, G.Z. Tu, L.B. Ma, N. Liao, X.J. Xu. *Tetrahedron*, **56**, 8915–8920 (2000).
[16] R. Roscher, J.P. Steffen, M. Herderich, W. Schwab, P. Schreier. *J. Agric. Food Chem.*, **44**, 1626–1629 (1996).
[17] F. Camps, J. Coll, A. Cortel. *Chem. Lett.*, 1313–1316 (1982).
[18] M.P. Calcagno, F. Camps, J. Coll, E. Melé, F. Sánchez-Baeza. *Tetrahedron*, **51**, 12119–12126 (1995).
[19] H. Hikino, K. Mohri, T. Okuyama, T. Takemoto. *Steroids*, **28**, 649–653 (1976).
[20] H. Hikino, T. Okuyama, S. Arihara, Y. Hikino, T. Takemoto, H. Mori, K. Shibata. *Chem. Pharm. Bull.*, **23**, 1458–1479 (1975).
[21] H. Hikino, Y. Hikino, K. Nomoto, T. Takemoto. *Tetrahedron*, **24**, 4895–4906 (1968).