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THREE PHYTOECDYSTEROIDS FROM *Sagina japonica* AND POTENTIAL BIOTRANSFORMING PATHWAYS OF JAPONICONE

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In continuation of the series of studies on the chemical components of the whole plant of Sagina japonica *Ohwi, another two phytoecdysteroids named 22,25-epoxy-24-methylene-2,3,14,20-tetrahydrocholest-7-en-6-one, or japonicone (**1**) and shidasterone (**2**), along with the previously reported compound 20-hydroxyecdysone (**3**), have been isolated on the basis of polyspectroscopic methods (^{1}H NMR, ^{13}C NMR, HMQC, HMBC, MS, and IR). The heteronuclear multiple bond coherence (HMBC) data of shidasterone (**2**) was supplemented for first time. Potential biotransforming pathways of japonicone (**1**) were discussed.*

Keywords: *Sagina japonica* Ohwi, phytoecdysteroid, shidasterone, japonicone, biotransformation.

Sagina japonica Ohwi (Caryophyllaceae) is a Chinese folk herb used for clearing up toxic heat, curing laccol, and drawing out pus [1]. It is distributed in Yunnan Province, in the Changjiang River and Huanghe River Valley area in China. In continuation of our study of the chemical components from the whole plants [2–6], two phytoecdysteroids were isolated from the EtOAc portion of the whole plant extract, along with one reported compound 20-hydroxyecdysone (**3**). Their structures were characterized as 22,25-epoxy-24-methylene-2,3,14,20-tetrahydrocholest-7-en-6-one, or japonicone (**1**) and shidasterone (**2**), and 20-hydroxyecdysone (**3**) by spectroscopic methods.

Phytoecdysteroids are one type of important natural compounds, according to L. Disan. Phytoecdysteroids are present predominantly in *Sagina* (Caryophyllaceae) [7], and the biosynthesis of 20-hydroxyecdysone (**3**) was referred to in some papers [8, 9], but the biotransformations of other compounds, such as makisterone and shidasterone, are enigmatic till now, so the potential biotransformations of japonicone (**1**) will be discussed here.

Compound **1**, obtained as colorless needles, gave a quasi-molecular ion peak at *m/z* 473 $[M - H]^+$ in the negative fast-atom bombardment (FAB-MS). The high-resolution electrospray ionization spectra (HR-ESI-MS) of **1** indicated a molecular formula $C_{28}H_{42}O_6$, which was derived from the molecular ion peak at *m/z* 497.2959 ($[M + Na]^+$, calcd 497.2936 $[M + Na]^+$) and confirmed by the ^{13}C NMR spectrum. In the IR spectrum, it showed strong absorption of hydroxyl groups at 3427 and 1059 cm^{-1} and characteristic absorption of the carboxylic group at 1645 cm^{-1} and the terminal olefinic group at 879 cm^{-1}. The ^{1}H NMR spectrum of **1** showed the present of two tertiary methyl groups [δ 1.04 (3H, s, H-18), 1.41 (3H, s, H-19)], a 25-methyl-Δ^{24}-sterol side chain [1.07 (3H, s, H-21), 4.09 (1H, m, H-22), 2.64 (1H, m, H-23), 2.48 (1H, m, H-23), 1.38 (3H, s, H-26), 1.32 (3H, s, H-27)], two oxygenated methine protons [4.17 (1H, br.s, H-2), 4.11 (1H, br.d, J = 11.56, H-3)], an olefinic proton [6.25 (1H, br.s, H-7)], and two terminal olefinic protons [4.87 (1H, s, H-28), 4.81 (1H, s, H-28)]. The ^{13}C NMR (DEPT) spectrum (Table 1) of **1** contained 28 signals (C × 8, CH × 7, CH_2 × 8, CH_3 × 5). The signals were similar to those of compound **3** except for the signal at δ 157.67 (s, C-24). In addition, the key correlations of compound **1** from the heteronuclear multiple bond coherence (HMBC) and rotating frame Overhauser effect spectroscopy spectra (ROESY) are shown in Fig. 1. The stereochemistry of compound **1** was determined by comparison of the ^{1}H and ^{13}C NMR data of compound **3** [10].

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TABLE 1. ^{13}C NMR (DEPT) Data for **1**, **2**, and **3** in C_5D_5N

C atom	1	2	3	C atom	1	2	3
1	38.68 (t)	38.68 (t)	38.72 (t)	15	32.49 (t)	32.50 (t)	32.08 (t)
2	68.16 (d)	68.17 (d)	68.21 (d)	16	21.05 (t)	21.11 (t)	21.18 (t)
3	68.11 (d)	68.11 (d)	68.13 (d)	17	51.41 (d)	51.47 (d)	50.17 (d)
4	32.49 (t)	32.50 (t)	32.48 (t)	18	17.94 (q)	17.90 (q)	17.94 (q)
5	51.47 (s)	51.42 (d)	51.44 (d)	19	24.45 (q)	24.46 (q)	24.51 (q)
6	203.36 (s)	203.44 (s)	203.57 (s)	20	75.23 (s)	75.58 (s)	77.64 (s)
7	121.74 (d)	121.71 (d)	121.73 (d)	21	21.05 (q)	21.21 (q)	21.74 (q)
8	165.96 (s)	166.08 (s)	166.16 (s)	22	81.84 (d)	80.43 (d)	76.96 (d)
9	34.50 (d)	34.47 (d)	34.52 (d)	23	34.85 (t)	28.30 (t)	27.51 (t)
10	38.04 (s)	38.03 (s)	38.03 (s)	24	157.67 (s)	38.98 (t)	42.66 (t)
11	21.78 (t)	21.73 (t)	21.54 (t)	25	81.84 (s)	84.97 (s)	69.69 (s)
12	31.73 (t)	31.71 (t)	31.81 (t)	26	28.92 (q)	27.68 (q)	30.05 (q)
13	47.63 (s)	47.69 (s)	48.18 (s)	27	27.68 (q)	28.83 (q)	30.15 (q)
14	84.21 (s)	84.18 (s)	84.28 (s)	28	103.42 (t)		

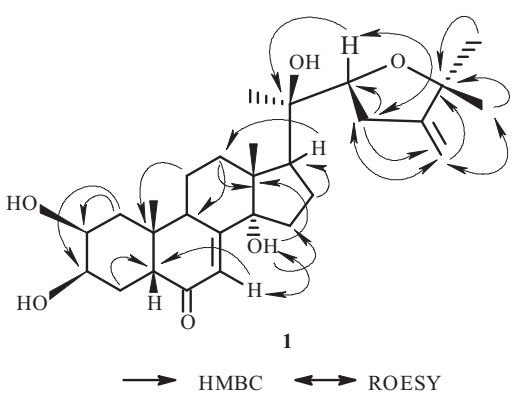

Fig. 1. HMBC and ROESY key correlations of japonicone (**1**).

From all of the data above, the structure of **1** was therefore deduced to be as shown in Fig. 1. It was named japonicone, or 22,25-epoxy-24-methylene-2,3,14,20-tetrahydrocholest-7-en-6-one according to IUPAC rules.

Compound **2**, $C_{27}H_{42}O_6$, showed the presence of five methyls, of which two tertiary methyls were included, three methine protons attached to two oxygen-bearing carbons, and one proton attached to one double bond in the ^1H NMR spectrum. Its ^{13}C NMR spectrum contained 27 peaks (Table 1). Compound **3**, $C_{27}H_{44}O_7$, also showed the presence of five methyls, of which two tertiary methyls were included, three methine protons attached to two oxygen-bearing carbons, and one proton attached to one double bond in the ^1H NMR spectrum. Its ^{13}C NMR spectrum contained 27 peaks (Table 1). Based upon spectroscopic (MS, IR, ^1H and ^{13}C NMR) and physical data comparison with the literature, compounds **2** and **3** are known compounds that were characterized as shidasterone (**2**) [10] and ecdysterone (**3**) [11]. Shidasterone (**2**) showed intense insect moulting hormone activity and antitumor activity [10, 12, 13], but there are no two-dimension NMR data of this compound in the previous literature, to the best of our knowledge. Here, the key correlations of it are as follows: HMBC: 1C-2H, 1C-5H, 2H-3C, 4H-3C, 5H-4C, 5H-3C, 5H-19C, 5H-6C, 5H-7C, 5H-9C, 7H-9C, 9H-10C, 9H-19C, 9H-11C, 9H-22C, 12H-11C, 12C-17H, 13C-17H, 14C-17H, 14C-7H, 14OH-15C, 15H-16C, 22H-21C, 23H-22C, 23H-25C, 27H-25C, 26H-25C, 20H-24C.

Because of the chemotaxonomic, chemoecological, and bioactive significance of phytoecdysteroids [7, 9, 14], two potential biotransformation pathways of compound **1** are discussed here (Fig. 2). One pathway is as follows: makisterone A (**4**) is methylated at C24 from 20-hydroxyecdysone (**3**) first, then 24(28)-dehydromakisterone A (**5**) is dehydrogenated at C24 and C28, and finally japonicone (**1**) is formed by intramolecular dehydration of 24(28)-dehydromakisterone A(**5**) at C22 and C25. Another way is as follows: shidasterone (**2**) is intramolecularly dehydrated from 20-hydroxyecdysone (**3**) first, then 24(28)-methylshidasterone A (**6**) is methylated at C24, and finally japonicone (**3**) is formed by dehydrogenation at C24 and C28. The base peak of 475 and other fragment peaks (unpublished data) in the FAB-MS spectrum were assumed to belong to 24(28)-methylshidasterone A (**6**), and besides the three identified compounds **1–3** by polyanalysis methods, all of these may indicate that japonicone (**1**) is transformed in *Sagina japonica* according to the second pathway primarily.

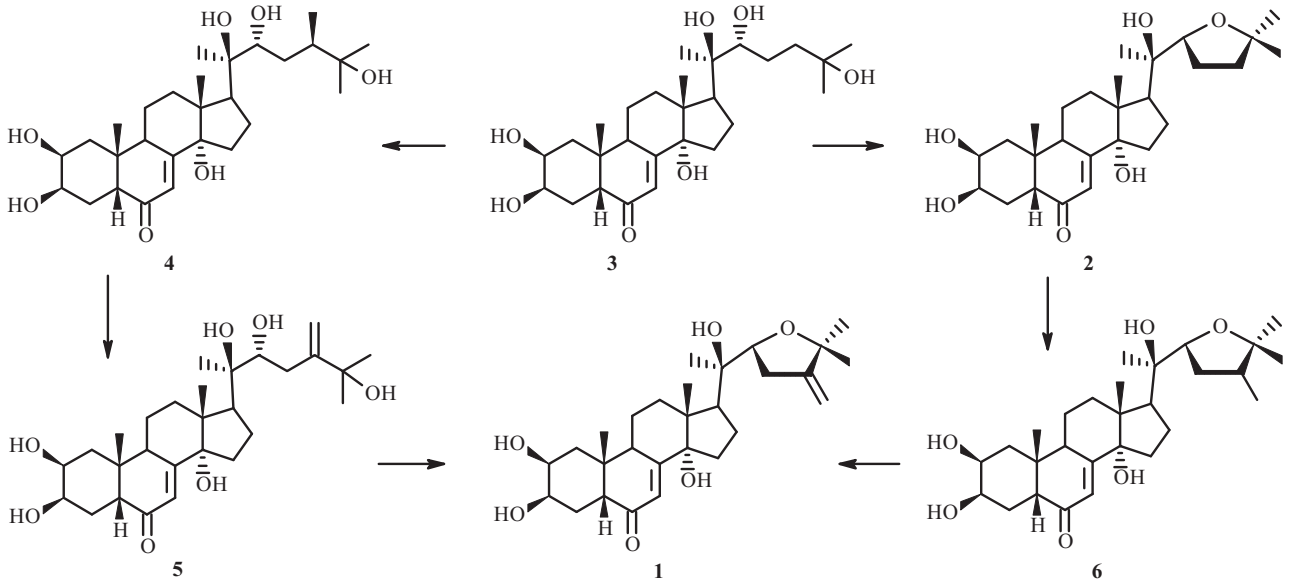

Fig. 2. Two potential biotransforming pathways of japonicone (**1**).

EXPERIMENTAL

General Methods. Melting points were obtained on an XRC-1 apparatus and were uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-400 spectrometer. The chemical shifts δ are given in ppm relative to TMS as an internal standard, and the coupling constants are given in Hz. The multiplicity of ^{13}C NMR was determined as DEPT spectra. Two-dimensional (2D) spectra were obtained with a Bruker DRX-500 instrument. Fast-atom bombardment mass spectrometry (FAB-MS) was recorded on a VG AutoSpec 3000 mass spectrometer. Infrared (IR) spectra were obtained with a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets.

Column chromatography (CC) was performed over silica Gel (200–300 and 230–400 mesh), LiChroprep RP-8 gel (40–63 μm), and Sephadex LH-20 gel (25–100, Pharmacia). Thin Layer chromatography (TLC) was carried out on plates precoated with Merck RP-18 and silica gel (Qingdao Marine Chemical Ltd., People′s Republic of China).

Extraction and Isolation. The air-dried powered whole plants of *S. japonica* Ohwi (21.0 kg) were extracted with 95% ethanol under reflux for three times (3 h, 1 h, and 1 h, respectively). The combined extract was concentrated under reduced pressure to furnish a residue, which was suspended in water and extracted with petroleum ether (60–90°C), EtOAc, and *n*-BuOH successively. The EtOAc portion was evaporated *in vacuo* to dryness to afford a fraction (620 g) that was desugarized on D101 macroporous resin eluted with aqueous MeOH (0:1–8:2). A quarter of the 65% MeOH eluate (100.0 × 1/4 g) was successively subjected to CC over silica gel (200–300 mesh) eluted with $CHCl_3$–MeOH gradient to afford fractions 1–6. Fraction 1 was chromatographed over silica gel using $CHCl_3$–MeOH as the eluant to furnish compounds **2** (100 mg) and **3** (80 mg). Fraction 3 was subjected to CC over Sephadex LH-20, RP-18, and MCI-gel CHP 20P eluted with MeOH–H_2O (45–80%) to afford compound **1** (1.6 g) with a yield of 0.03%.

Japonicone{22,25-epoxy-24-methylene-2,3,14,20-tetrahydrocholest-7-en-6-on} (1). Colorless needles, mp 231–232°C; $[\alpha]_D^{18}$ +75.8° (*c* 0.13, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 224 (1.32). IR (KBr, ν_{max}, cm^{-1}): 3427, 2930, 1645, 1059, 879 (characteristic peak of RCH=CH_2). FAB-MS *m/z* (relative intensities): 473 $[M - 1]^+$ (100), 455 $[M^+ - H_2O]$ (10), 361 (20), 318 (18), 125 (15). HR-ESI-MS *m/z*: found: 497.2959 $[M + Na]^+$, calcd: 497.2936 $[M + Na]^+$, calcd for $C_{28}H_{42}O_6Na$. ^{1}H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 2.10 (1H, m, H_{eq}-1), 1.92 (1H, m, H_{ax}-1), 4.17 (1H, br.s, H-2), 4.11 (1H, br.d, J = 11.56, H-3), 2.14 (1H, m, H_{ax}-4), 2.67 (1H, m, H_{eq}-4), 3.02 (1H, dd, J = 2.24, 11.03, H-5), 6.25 (1H, br.s, H-7), 3.61 (1H, t, J = 10.24, H-9), 1.91 (1H, m, H_{eq}-11), 1.75 (1H, m, H_{ax}-11), 2.08 (1H, m, H_{eq}-12), 1.92 (1H, m, H_{ax}-12), 6.36 (1H, s, OH-14), 2.13 (1H, m, H_{eq}-15), 1.94 (1H, m, H_{ax}-15), 2.25 (1H, m, H_{eq}-16), 2.10 (1H, m, H-16), 2.92 (1H, t, J = 7.21, H-17), 1.04 (3H, s, H-18), 1.41 (3H, s, H-19), 1.07 (3H, s, H-21), 4.09 (1H, m, H-22), 2.64 (1H, m, H-23), 2.48 (1H, m, H-23), 1.38 (3H, s, H-26), 1.32 (3H, s, H-27), 4.87 (1H, s, H-28), 4.81 (1H, s, H-28); for ^{13}C NMR, see Table 1.

Shidasterone (2). Colorless crystals, mp 258–262°C, $[\alpha]_D^{18}$ +58.96° (*c* 0.36, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 205 (1.87). FAB-MS *m/z* (relative intensities): 461 $[M - 1]^+$ (100), 445 (50), 318, 287, 173, 125. ^{1}H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 2.16 (1H, m, H_{eq}-1), 1.97 (1H, m, H_{ax}-1), 4.25 (1H, br.s, H-2), 4.19 (1H, m, H-3), 2.04 (1H, m, H_{ax}-4), 2.14 (1H, m, H_{eq}-4), 3.02 (1H, dd, J = 2.52, 10.80, H-5), 6.21 (1H, s, H-7), 3.61 (1H, m, H-9), 1.89 (1H, m, H_{eq}-11), 1.71 (1H, m, H_{ax}-11), 2.64 (2H, m, H-12), 1.86 (2H, m, H-15), 2.07 (2H, m, H-16), 2.86 (1H, dd, J = 8.56, 8.28, H-17), 1.07 (3H, s, H-18), 1.09 (3H, s, H-19), 1.40 (3H, s, H-21), 4.09 (1H, dd, J = 8.08, 7.04, H-22), 2.09 (1H, m, H-23), 1.81 (1H, m, H-23), 1.63 (2H, m, H-24), 1.22 (3H, s, H-26), 1.20 (3H, s, H-27); for ^{13}C NMR, see Table 1.

20-Hydroxyecdysone (3). Colorless needles, mp 246–248°C. FAB-MS *m/z*: 479 $[M - 1]^+$ (100), 328, 295, 273, 183, 125. ^{1}H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 2.16 (1H, m, H_{eq}-1), 1.95 (1H, m, H_{ax}-1), 3.94 (1H, br.s, H-2), 3.84 (1H, m, H-3), 2.12 (1H, m, H_{ax}-4), 2.36 (1H, m, H_{eq}-4), 3.16 (1H, dd, J = 2.52, 10.16, H-5), 5.80 (1H, s, H-7), 3.34 (1H, m, H-9), 1.88 (1H, m, H_{eq}-11), 1.71 (1H, m, H_{ax}-11), 2.36 (2H,m, H-12), 1.78 (2H, m, H-15), 1.95 (2H, m, H-16), 2.36 (1H, dd, J = 8.56, 8.28, H-17), 0.95 (3H, s, H-18), 0.88 (3H, s, H-19), 1.19 (3H, s, H-21), 3.93 (1H, m, H-22), 1.97 (1H, m, H-23), 1.77 (1H, m, H-23), 1.61 (2H, m, H-24), 1.19 (3H, s, H-26), 1.18 (3H, s, H-27); for ^{13}C NMR, see Table 1.

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