

## Terpenoids and Flavonoids from *Arenaria kansuensis*<sup>1)</sup>

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A new steroid, 22,23-dihydrospinasterol palmitate was isolated from the whole plants of *Arenaria kansuensis* (Caryophyllaceae) and its structure was determined by chemical and spectroscopic means. 22,23-Dihydrospinasterol, 22,23-dihydrospinasterone, ergosterol-5,8-peroxide, 24-methylene-22,23-dihydrolanosterol, zeorin, fernenone,  $\beta$ -sitosterol 3 $\beta$ -O- $\beta$ -D-glucopyranoside, tricin, (+)-isoscoparin and (–)-isoscoparin were also isolated.

**Keywords** *Arenaria kansuensis*; Caryophyllaceae; steroid; triterpenoid; flavonoid; 22,23-dihydrospinasterol palmitate

The whole plants of *Arenaria kansuensis* MAXIM. (雪靈芝, Chinese name: Xue ling zhi) (Caryophyllaceae), a very important Chinese folk medicine, have been used to treat influenza, lung inflammation, jaundice and rheumatism.<sup>2)</sup> Previously,<sup>1)</sup> we reported the isolation of four new  $\beta$ -carboline alkaloids named arenarins A—D. In a continuation of our chemical studies on this plant, we isolated five steroids, three triterpenoids and three flavonoids.

Compounds **1**, **2**, **3**, **10** and **11** were shown to be the known 22,23-dihydrospinasterol,<sup>3)</sup> 22,23-dihydrospinasterone,<sup>4)</sup> ergosterol-5,8-peroxide,<sup>5)</sup> (+)-isoscoparin<sup>6)</sup> and (–)-isoscoparin,<sup>7)</sup> respectively, based on their spectral data. Compound **4**, **5**, **6**, **8** and **9** were identified as 24-methylene-22,23-dihydrolanosterol,<sup>8)</sup> zeorin,<sup>9)</sup> fernenone,<sup>10)</sup>  $\beta$ -sitosterol 3 $\beta$ -O- $\beta$ -D-glucopyranoside<sup>11)</sup> and tricin<sup>12)</sup> by direct comparison with respective authentic samples.

Compound **7**, a new steroid, was obtained as amorphous powder with  $[\alpha]_D^{25} + 4.4^\circ$  (CHCl<sub>3</sub>) and showed an ester absorption (1740 and 1180 cm<sup>-1</sup>) in the infrared (IR) spectrum. From observation of the high-resolution mass spectrum (HRMS), the molecular formula was concluded to be C<sub>45</sub>H<sub>80</sub>O<sub>2</sub>. Alkaline hydrolysis of **7** gave 22,23-dihydrospinasterol (**1**) and palmitic acid which were identified by gas liquid chromatographic (GLC) analysis. Thus, compound **7** was identified as 22,23-dihydrospinasterol palmitate.

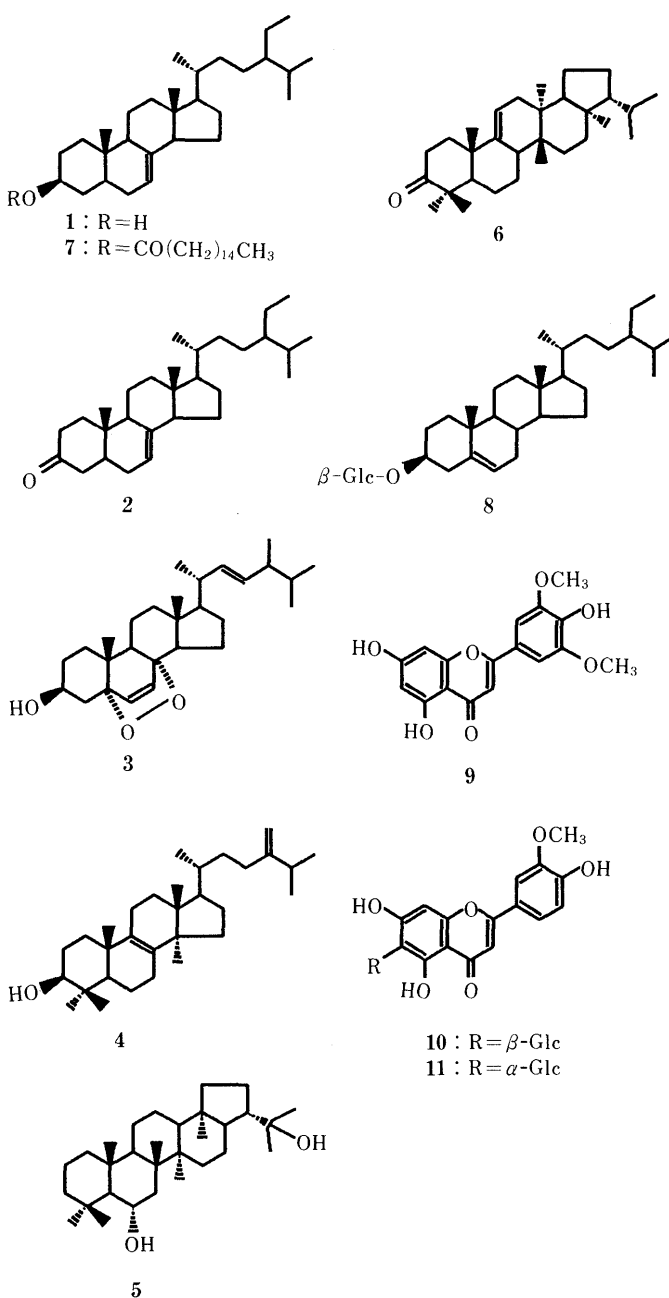
This is the first report on the isolation of compounds **2**—**11** from genus *Arenaria* (Caryophyllaceae).

### Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The ultraviolet (UV) and IR spectra were recorded with Hitachi 340 and Hitachi 260-30 spectrophotometers, respectively. The MS, HRMS and fast-atom bombardment (FAB)-MS were measured on JEOL JMS D-300 and JEOL DX-303 mass spectrometers, respectively. The proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra were measured with a JEOL JNM GX-400 (<sup>1</sup>H at 400 MHz) spectrometer. Chemical shifts are expressed in  $\delta$  (ppm) down-field from tetramethylsilane as an internal standard, and coupling constants (*J*) in Hz. Column chromatography was carried out on silica gel (BWH-820 MH, Fuji Davison). Optical rotations were determined on a JASCO DIP-4 digital polarimeter. High-performance liquid chromatography (HPLC) was carried out on an octadecyl silica column (Capcell pak C<sub>18</sub> Shiseido, 10 mm i.d.  $\times$  250 mm, solvent: MeOH–H<sub>2</sub>O (3:2)). GLC analyses were carried out on a Hitachi 163 gas liquid chromatograph with a flame ionization detector using a stainless steel column (3 mm i.d.  $\times$  1 m) packed with 2% SE-30 on Chromosorb-W (60–80 mesh). Nitrogen was used as carrier gas at a flow rate of 35 ml/min.

**Isolation** The fractionation of MeOH extract from *Arenaria kansuensis* was described in a previous report.<sup>1)</sup> The ether fraction (15.0 g) was chromatographed on silica gel to give 22,23-dihydrospinasterol (**1**, 70 mg),

22,23-dihydrospinasterone (**2**, 4 mg), ergosterol-5,8-peroxide (**3**, 20 mg), 24-methylene-22,23-dihydrolanosterol (**4**, 20 mg), zeorin (**5**, 70 mg), fernenone (**6**, 10 mg) and 22,23-dihydrospinasterol palmitate (**7**, 8 mg). The



chloroform extract (6.6 g) was chromatographed with silica gel to give  $\beta$ -sitosterol 3 $\beta$ -*O*- $\beta$ -D-glucopyranoside (**8**, 20 mg) and tricin (**9**, 10 mg). *n*-Butanol fraction (6.6 g) was chromatographed on silica gel and then purified by HPLC to give (+)-isoscoparin (**10**, 20 mg) and (-)-isoscoparin (**11**, 26 mg).

**22,23-Dihydrospinasterol (1)**<sup>3)</sup> Colorless needles (hexane-ethyl acetate), mp 153–154 °C,  $[\alpha]_D^{26} + 4.5^\circ$  ( $c=0.2$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3450, 2960, 2860, 1642, 1465, 1380, 1100, 1050. MS  $m/z$ : 414 (M<sup>+</sup>, 100%), 399 (27), 314 (29), 271 (73), 255 (68). HRMS: Calcd for C<sub>29</sub>H<sub>50</sub>O,  $m/z$  414.3870. Found:  $m/z$  414.3862. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.54, 0.80 (each 3H, s), 0.82, 0.93, 0.97 (each 3H, d,  $J=7$  Hz), 0.85 (3H, t,  $J=7$  Hz), 3.59 (1H, m), 5.16 (1H, dd,  $J=5$ , 2 Hz).

**22,23-Dihydrospinasterone (2)**<sup>4)</sup> Colorless needles (hexane-ethyl acetate), mp 159–160 °C,  $[\alpha]_D^{26} + 26.1^\circ$  ( $c=0.6$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3450, 2990, 2880, 1708, 1470, 1390. MS  $m/z$ : 412 (M<sup>+</sup>, 100%), 397 (25), 312 (29), 271 (66), 244 (20). HRMS: Calcd for C<sub>29</sub>H<sub>48</sub>O,  $m/z$  412.3705. Found:  $m/z$  412.3687. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.57, 1.01 (each 3H, s), 0.82, 0.85, 0.94 (each 3H, d,  $J=7$  Hz), 0.87 (3H, t,  $J=6$  Hz), 5.19 (1H, dd,  $J=4$ , 2 Hz).

**Ergosterol-5,8-peroxide (3)**<sup>5)</sup> Colorless needles (hexane-ethyl acetate), mp 179–180 °C,  $[\alpha]_D^{26} - 19.6^\circ$  ( $c=0.5$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3540, 3400, 2950, 1620, 1460, 1380, 1040, 960. MS  $m/z$ : 428 (M<sup>+</sup>, 2%), 396 (100), 376 (10), 363 (36), 337 (14). HRMS: Calcd for C<sub>28</sub>H<sub>44</sub>O<sub>3</sub>,  $m/z$ : 428.3402. Found:  $m/z$  428.3295. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.82, 0.88 (each 3H, s), 0.82, 0.83, 0.91, 1.00 (each 3H, d,  $J=7$  Hz), 5.17, 5.22 (each 1H, dd,  $J=16$ , 7 Hz), 6.24, 6.50 (each 1H, d,  $J=9$  Hz).

**24-Methylene-22,23-dihydrolanosterol (4)**<sup>8)</sup> Colorless needles (hexane-ethyl acetate), mp 161–162 °C,  $[\alpha]_D^{26} + 47.3^\circ$  ( $c=0.5$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3500, 2950, 2870, 2640, 1460, 1377, 1260, 1100, 1030. MS  $m/z$ : 440 (M<sup>+</sup>, 46%), 425 (100), 411 (31), 393 (13), 341 (12). HRMS: Calcd for C<sub>31</sub>H<sub>52</sub>O,  $m/z$ : 440.4018. Found:  $m/z$ : 440.4029. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.70, 0.81, 0.88, 0.98, 1.00 (3H, s), 0.92, 1.02, 1.03 (each 3H, d,  $J=7$  Hz), 3.24 (1H, dd,  $J=12$ , 4 Hz), 4.69 (1H, d,  $J=2$  Hz), 4.71 (1H, br s).

Acetylation of **4**: Compound **4** (10 mg) was acetylated with Ac<sub>2</sub>O (0.2 ml) and pyridine (0.2 ml) at room temperature for 10 h to give monoacetate of **4** (10 mg), mp 130–132 °C. MS  $m/z$ : 482 (M<sup>+</sup>, 51%), 467 (100), 439 (4), 407 (67), 383 (8). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3440, 2950, 1738, 1250. This compound was identified by direct comparison (mixed melting point determination, thin layer chromatography (TLC) behavior, IR and <sup>1</sup>H-NMR spectra) by 24-methylene-22,23-dihydrolanosterol-3 $\beta$ -acetate.

**Zeorin (5)**<sup>9)</sup> Colorless needles (hexane-ethyl acetate), mp 233–234 °C,  $[\alpha]_D^{26} + 48.0^\circ$  ( $c=0.2$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3350, 2950, 1630, 1460, 1390, 1160. MS  $m/z$ : 444 (M<sup>+</sup>, 11%), 426 (13), 383 (7), 357 (6), 207 (80), 189 (100). HRMS: Calcd for C<sub>30</sub>H<sub>52</sub>O<sub>2</sub>,  $m/z$  444.3967. Found:  $m/z$  444.3990. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.76, 0.87, 0.98, 1.02, 1.04, 1.16, 1.18, 1.21 (each 3H, s), 3.96 (1H, m). This compound was identified by direct comparison (mixed melting point determination, TLC behavior, IR and <sup>1</sup>H-NMR spectra) with zeorin.

**Fernenone (6)**<sup>10)</sup> Colorless plates (hexane-ethyl acetate), mp 200–201 °C,  $[\alpha]_D^{26} - 38.4^\circ$  ( $c=0.2$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 2980, 2865, 1702, 1470, 1380, 1118. MS  $m/z$ : 424 (M<sup>+</sup>, 47%), 409 (83), 367 (2), 339 (7), 271 (20), 257 (100). HRMS: Calcd for C<sub>30</sub>H<sub>48</sub>O,  $m/z$  424.3705. Found:  $m/z$  424.3687. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.75, 0.76, 0.79, 1.04, 1.12, 1.30 (each 3H, s), 0.83, 0.89 (each 3H, d,  $J=6$  Hz), 5.36 (1H, m). This compound was identified by direct comparison (mixed melting point determination, TLC behavior, IR and <sup>1</sup>H-NMR spectra) with fernenone.

**22,23-Dihydrospinasterol Palmitate (7)** Colorless amorphous powder,  $[\alpha]_D^{26} + 4.4^\circ$  ( $c=0.3$ , CHCl<sub>3</sub>). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 2910, 2850, 1740, 1460, 1390, 1180, 1100. MS  $m/z$ : 652 (M<sup>+</sup>, 99%), 638 (20), 511 (11), 396 (54), 381 (30), 255 (94), 229 (40), 213 (56), 43 (100). HRMS: Calcd for C<sub>45</sub>H<sub>80</sub>O<sub>2</sub>,  $m/z$  652.6158. Found:  $m/z$  652.6168. <sup>1</sup>H-NMR ( $\delta$  in CDCl<sub>3</sub>): 0.54, 0.85 (each 3H, s), 0.82, 0.83, 0.93 (each 3H, d,  $J=7$  Hz), 0.85, 0.88 (each 3H, t,  $J=7$  Hz), 1.27 (26H, m), 2.27 (2H, t,  $J=7$  Hz), 4.71 (1H, m), 5.15 (1H, dd,  $J=6$ , 2 Hz).

**Hydrolysis of 7** A solution of **7** (5 mg) in MeOH (2 ml) containing 5% KOH (0.5 ml) was refluxed for 1 h. The reaction mixture was acidified with HCl and extracted with ether to give sterol and fatty acid, which were shown to be identical with **1** and palmitic acid by GLC.

**$\beta$ -Sitosterol 3 $\beta$ -*O*- $\beta$ -D-Glucopyranoside (8)**<sup>11)</sup> Amorphous powder, mp

295–296 °C. IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3440, 2960, 2900, 1480, 1380, 1180, 1088, 1040. MS  $m/z$ : 414 [(M-Glc)<sup>+</sup>, 8%], 396 (100), 382 (13), 303 (8), 288 (21), 255 (29). <sup>1</sup>H-NMR ( $\delta$  in DMSO-*d*<sub>6</sub>): 0.50, 0.74 (each 3H, s), 0.80, 0.91, 0.94 (each 3H, d,  $J=7$  Hz), 0.82 (1H, t,  $J=8$  Hz), 3.55 (1H, m), 4.22 (1H, d,  $J=8$  Hz, Glc-H-1), 5.13 (1H, m). This compound was identified by direct comparison (mixed melting point determination, TLC behavior, IR and <sup>1</sup>H-NMR spectra) with  $\beta$ -sitosterol 3 $\beta$ -*O*- $\beta$ -D-glucopyranoside.

**Tricin (9)**<sup>12)</sup> Yellow needles (MeOH), mp 297–298 °C (dec.). UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 270 (4.09), 348 (4.29). UV  $\lambda_{\max}^{\text{MeOH} + \text{AlCl}_3}$  nm (log  $\epsilon$ ): 278 (4.13), 308 (3.84), 392 (4.28). UV  $\lambda_{\max}^{\text{MeOH} + \text{NaOAc}}$  nm (log  $\epsilon$ ): 276 (4.18), 324 (4.09), 360 (4.16). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3430, 1655, 1623, 1610, 1585, 1265, 1160, 1115, 1050. MS  $m/z$ : 330 (M<sup>+</sup>, 100%), 302 (7), 259 (5), 213 (5), 178 (8). HRMS: Calcd for C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>,  $m/z$  330.0740. Found:  $m/z$  330.0793. <sup>1</sup>H-NMR ( $\delta$  in DMSO-*d*<sub>6</sub>): 3.89 (6H, s, -OCH<sub>3</sub> × 2), 6.21 (1H, d,  $J=2$  Hz, H-6), 6.56 (1H, d,  $J=2$  Hz, H-8), 6.98 (1H, s, H-3), 7.33 (2H, s, H-2', 6'), 12.97 (1H, s, 5-OH). This compound was identified by direct comparison (mixed melting point determination and IR and <sup>1</sup>H-NMR spectra) with tricrin.

**(+)-Isoscoparin (10)**<sup>6)</sup> Yellow needles (MeOH), mp 208–210 °C (dec.),  $[\alpha]_D^{21} + 16.2^\circ$  ( $c=0.2$ , MeOH). UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 280 (4.13), 350 (4.18). UV  $\lambda_{\max}^{\text{MeOH} + \text{AlCl}_3}$  nm (log  $\epsilon$ ): 267 (4.10), 310 (3.99), 365 (4.04), 388 (4.01). UV  $\lambda_{\max}^{\text{MeOH} + \text{NaOAc}}$  nm (log  $\epsilon$ ): 282 (3.91), 375 (3.84). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3400, 1620, 1290, 1202, 1080. FAB-MS  $m/z$ : 485 (M+Na)<sup>+</sup>, 463 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR ( $\delta$  in DMSO-*d*<sub>6</sub>): 3.89 (3H, s, -OCH<sub>3</sub>), 4.64 (1H, d,  $J=10$  Hz, Glc-H-1), 6.40 (1H, s, H-8), 6.70 (1H, s, H-3), 6.93 (1H, d,  $J=8$  Hz, H-5'), 7.49 (2H, m, H-2', 6'), 13.45 (1H, br s, 5-OH).

**(-)-Isoscoparin (11)**<sup>7)</sup> Yellow needles (MeOH), mp 202–204 °C (dec.),  $[\alpha]_D^{21} - 24.8^\circ$  ( $c=0.5$ , MeOH). UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 270 (3.75), 344 (3.86). UV  $\lambda_{\max}^{\text{MeOH} + \text{AlCl}_3}$  nm (log  $\epsilon$ ): 264 (3.73), 276 (3.77), 296 (3.55), 366 (3.88), 386 (4.01). UV  $\lambda_{\max}^{\text{MeOH} + \text{NaOAc}}$  (log  $\epsilon$ ): 271 (3.82), 322 (3.60), 372 (3.77). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3380, 1620, 1295, 1204, 1080. FAB-MS  $m/z$ : 485 (M+Na)<sup>+</sup>, 463 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR ( $\delta$  in DMSO-*d*<sub>6</sub>): 3.89 (3H, s, -OCH<sub>3</sub>), 4.73 (1H, d,  $J=10$  Hz, Glc-H-1), 6.40 (1H, s, H-8), 6.70 (1H, s, H-3), 6.93 (1H, d,  $J=8$  Hz, H-5'), 7.49 (2H, m, H-2', 6').

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