Terpenoids and Flavonoids from *Arenaria kansuensis*[1)]

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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, β-sitosterol 3β-*O*-β-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. (雪霊芝, Chinese name: Xue ling zhi) (Caryophyllaceae), a very important Chinese folk medicine, have been used to treat influenza, lung inflammation, jaundice and rheumatism.[2)] Previously,[1)] we reported the isolation of four new β-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,[3)] 22,23-dihydrospinasterone,[4)] ergosterol-5,8-peroxide,[5)] (+)-isoscoparin[6)] and (−)-isoscoparin,[7)] respectively, based on their spectral data. Compound **4**, **5**, **6**, **8** and **9** were identified as 24-methylene-22,23-dihydrolanosterol,[8)] zeorin,[9)] fernenone,[10)] β-sitosterol 3β-*O*-β-D-glucopyranoside[11)] and tricin[12)] by direct comparison with respective authentic samples.

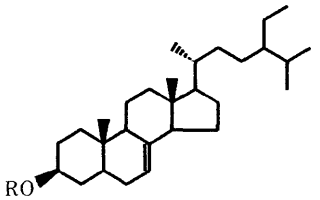
Compound **7**, a new steroid, was obtained as amorphous powder with $[\alpha]_D$ +4.4° ($CHCl_3$) and showed an ester absorption (1740 and 1180 cm^{-1}) in the infrared (IR) spectrum. From observation of the high-resolution mass spectrum (HRMS), the molecular formula was concluded to be $C_{45}H_{80}O_2$. 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).

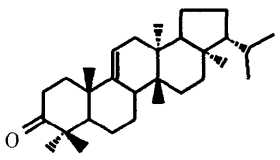
Experimental

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 (^{1}H-NMR) spectra were measured with a JEOL JNM GX-400 (^{1}H at 400 MHz) spectrometer. Chemical shifts are expressed in δ (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_{18} Shiseido, 10 mm i.d. × 250 mm, solvent: MeOH–H_2O (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. × 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.[1)] 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



1 : R=H
7 : R=CO(CH$_2$)$_{14}$CH$_3$



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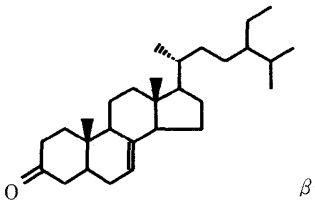

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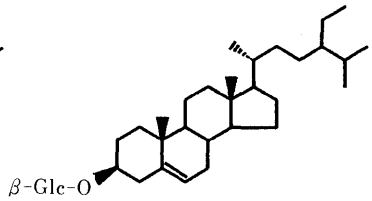

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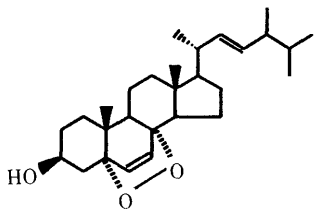

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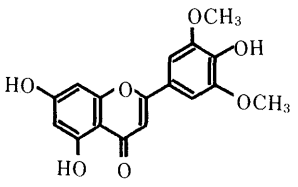

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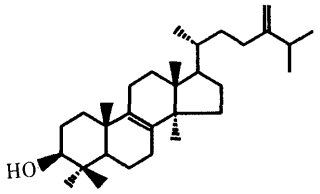

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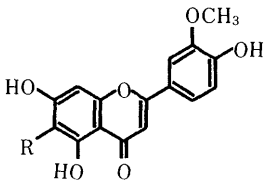
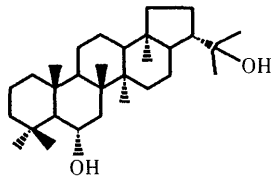


10 : R=β-Glc
11 : R=α-Glc



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chloroform extract (6.6 g) was chromatographed with silica gel to give β-sitosterol 3β-*O*-β-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)[3] Colorless needles (hexane–ethyl acetate), mp 153—154 °C, $[\alpha]_D^{26}$ +4.5° (*c*=0.2, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2960, 2860, 1642, 1465, 1380, 1100, 1050. MS *m/z*: 414 (M^+, 100%), 399 (27), 314 (29), 271 (73), 255 (68). HRMS: Calcd for $C_{29}H_{50}O$, *m/z* 414.3870. Found: *m/z* 414.3862. ^{1}H-NMR (δ in $CDCl_3$): 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)[4] Colorless needles (hexane–ethyl acetate), mp 159—160 °C, $[\alpha]_D^{26}$ +26.1° (*c*=0.6, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2990, 2880, 1708, 1470, 1390. MS *m/z*: 412 (M^+, 100%), 397 (25), 312 (29), 271 (66), 244 (20). HRMS: Calcd for $C_{29}H_{48}O$, *m/z* 412.3705. Found: *m/z* 412.3687. ^{1}H-NMR (δ in $CDCl_3$): 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)[5] Colorless needles (hexane–ethyl acetate), mp 179—180 °C, $[\alpha]_D^{26}$ −19.6° (*c*=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3540, 3400, 2950, 1620, 1460, 1380, 1040, 960. MS *m/z*: 428 (M^+, 2%), 396 (100), 376 (10), 363 (36), 337 (14). HRMS: Calcd for $C_{28}H_{44}O_3$, *m/z*: 428.3402. Found: *m/z* 428.3295. ^{1}H-NMR (δ in $CDCl_3$): 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)[8] Colorless needles (hexane–ethyl acetate), mp 161—162 °C, $[\alpha]_D^{26}$ +47.3° (*c*=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 2950, 2870, 2640, 1460, 1377, 1260, 1100, 1030. MS *m/z*: 440 (M^+, 46%), 425 (100), 411 (31), 393 (13), 341 (12). HRMS: Calcd for $C_{31}H_{52}O$, *m/z*: 440.4018. Found: *m/z*: 440.4029. ^{1}H-NMR (δ in $CDCl_3$): 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_2O (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^+, 51%), 467 (100), 439 (4), 407 (67), 383 (8). IR ν_{max}^{KBr} cm^{-1}: 3440, 2950, 1738, 1250. This compound was identified by direct comparison (mixed melting point determination, thin layer chromatography (TLC) behavior, IR and ^{1}H-NMR spectra) by 24-methylene-22,23-dihydrolanosterol-3β-acetate.

Zeorin (5)[9] Colorless needles (hexane–ethyl acetate), mp 233—234 °C, $[\alpha]_D^{26}$ +48.0° (*c*=0.2, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3350, 2950, 1630, 1460, 1390, 1160. MS *m/z*: 444 (M^+, 11%), 426 (13), 383 (7), 357 (6), 207 (80), 189 (100). HRMS: Calcd for $C_{30}H_{52}O_2$, *m/z* 444.3967. Found: *m/z* 444.3990. ^{1}H-NMR (δ in $CDCl_3$): 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 comarison (mixed melting point determination, TLC behavior, IR and ^{1}H-NMR spectra) with zeorin.

Fernenone (6)[10] Colorless plates (hexane–ethyl acetate), mp 200—201 °C, $[\alpha]_D^{26}$ −38.4° (*c*=0.2, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2980, 2865, 1702, 1470, 1380, 1118. MS *m/z*: 424 (M^+, 47%), 409 (83), 367 (2), 339 (7), 271 (20), 257 (100). HRMS: Calcd for $C_{30}H_{48}O$, *m/z* 424.3705. Found: *m/z* 424.3687. ^{1}H-NMR (δ in $CDCl_3$): 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 ^{1}H-NMR spectra) with fernenone.

22,23-Dihydrospinasterol Palmitate (7) Colorless amorphous powder, $[\alpha]_D^{26}$ +4.4° (*c*=0.3, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2910, 2850, 1740, 1460, 1390, 1180, 1100. MS *m/z*: 652 (M^+, 99%), 638 (20), 511 (11), 396 (54), 381 (30), 255 (94), 229 (40), 213 (56), 43 (100). HRMS: Calcd for $C_{45}H_{80}O_2$, *m/z* 652.6158. Found: *m/z* 652.6168. ^{1}H-NMR (δ in $CDCl_3$): 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.

β-Sitosterol 3β-*O*-β-D-Glucopyranoside (8)[11] Amorphous powder, mp 295—296 °C. IR ν_{max}^{KBr} cm^{-1}: 3440, 2960, 2900, 1480, 1380, 1180, 1088, 1040. MS *m/z*: 414 [$(M-Glc)^+$, 8%], 396 (100), 382 (13), 303 (8), 288 (21), 255 (29). ^{1}H-NMR (δ in DMSO-d_6): 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 ^{1}H-NMR spectra) with β-sitosterol 3β-*O*-β-D-glucopyranoside.

Tricin (9)[12] Yellow needles (MeOH), mp 297—298 °C (dec.). UV λ_{max}^{MeOH} nm (log ε): 270 (4.09), 348 (4.29). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 278 (4.13), 308 (3.84), 392 (4.28). UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 276 (4.18), 324 (4.09), 360 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3430, 1655, 1623, 1610, 1585, 1265, 1160, 1115, 1050. MS *m/z*: 330 (M^+, 100%), 302 (7), 259 (5), 213 (5), 178 (8). HRMS: Calcd for $C_{17}H_{14}O_7$, *m/z* 330.0740. Found: *m/z* 330.0793. ^{1}H-NMR (δ in DMSO-d_6): 3.89 (6H, s, $-OCH_3\times2$), 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 ^{1}H-NMR spectra) with tricin.

(+)-Isoscoparin (10)[6] Yellow needles (MeOH), mp 208—210 °C (dec.), $[\alpha]_D^{21}$ +16.2° (*c*=0.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 280 (4.13), 350 (4.18). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 267 (4.10), 310 (3.99), 365 (4.04), 388 (4.01). UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 282 (3.91), 375 (3.84). IR ν_{max}^{KBr} cm^{-1}: 3400, 1620, 1290, 1202, 1080. FAB-MS *m/z*: 485 $(M+Na)^+$, 463 $(M+H)^+$. ^{1}H-NMR (δ in DMSO-d_6): 3.89 (3H, s, $-OCH_3$), 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)[7] Yellow needles (MeOH), mp 202—204 °C (dec.), $[\alpha]_D^{21}$ −24.8° (*c*=0.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 270 (3.75), 344 (3.86). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 264 (3.73), 276 (3.77), 296 (3.55), 366 (3.88), 386 (4.01). UV $\lambda_{max}^{MeOH+NaOAc}$ (log ε). 271 (3.82), 322 (3.60), 372 (3.77). IR ν_{max}^{KBr} cm^{-1}: 3380, 1620, 1295, 1204, 1080. FAB-MS *m/z*: 485 $(M+Na)^+$, 463 $(M+H)^+$. ^{1}H-NMR (δ in DMSO-d_6): 3.89 (3H, s, $-OCH_3$), 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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