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Chemical constituents from the aerial part of *Sheareria nana* S. Moore

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ABSTRACT

Seventeen compounds were isolated from the aerial parts of Sheareria nana S. Moore. Their structures were elucidated by spectroscopic methods and by comparison of the reported spectral data. They consisted of two sterols (**1** and **12**), five triterpenoids (**2**–**6**), two lignanoids (**7** and **16**), two diterpenoids (**8** and **9**), two phenylpropanoids (**10** and **11**), three flavonoids (**13**, **15** and **17**) and one quinone (**14**). The taxonomic influence of these compounds in the position of this species within the Compositae family was discussed. As the result, the neo-clerodane diterpenes (soulidiol and soulidiol 18-O-β-D-glucoside), sterol (α-spinasterol-3-O-β-D-glucoside) and triterpenoids (urs-12-ene and olean-12-ene) might depict the taxonomic position for S. nana in Compositae and demonstrate the relationships between S. nana and some species of Astereae and Inuleae.

1. Subject and source

Sheareria genus (Compositae family), a monotypic genus endemic to China, contains only one species *Sheareria nana* S. Moore, which is distributed in the east, south and southwest parts of China as an annual herb (Li et al., 2008). The aerial part of *Sheareria nana* S. Moore, with its trivial name Xiaxucao in Chinese, is used to treat edema and headache (Zhonghua Bencao, 1999). In order to clarify the chemical constituents and the taxonomic character of this plant, a systematic phytochemical investigation was carried out, and the chemotaxonomic relationship was also discussed.

The dried aerial part of *S. nana* was collected in Yueyang, Hunan province of China in September, 2015, and was identified by Dr. Xin-Li Zhan, Spine and Osteopathy Ward, the First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, Guangxi Zhuang Autonomous Region, P.R.China. A voucher specimen was deposited under No. SN-20150901.

2. Previous work

No literature were found on the role of compounds in the chemotaxonomic of *S. nana*. A microcharacter study suggested that *S. nana* might be delimited within the Astereae (Li et al., 2008).

3. Present work

The air dried aerial parts of *S. nana* (5 kg) were extracted with aqueous EtOH (85%, v/v) at room temperature for three times, seven days per time. The combined solvent was filtered and concentrated under reduced pressure at 50 °C to yield a brown crude extract (0.6 kg). The extract was then suspended in water and partitioned successively with petroleum ether (PE, boiling point 60–90 °C), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and n–BuOH. All partitions were lyophilized to give PE (40 g), CH_2Cl_2 (72 g), EtOAc (85 g) and n–BuOH (106 g) extracts, respectively.

The PE fraction (35 g) was solved in PE–EtOAc mixed solvent and absorbed by silica gel (100 g), then evaporated to dryness. The mixture was subjected to a silica gel (100–200 mesh) column chromatography (CC) and eluted with a step gradient of PE–EtOAc (gradient, from 49:1 to 0:1, v/v) to yield five fractions (Fr. P_I–P_V). Fr. P_I (4 g) was subjected to a silica gel CC (200–300 mesh) with

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PE–EtOAc (gradient, from 39:1 to 1:1, v/v) as eluent to give three sub–fractions (sub-fraction P_{I}a–P_{I}c). Sub-fraction P_{I}b was isolated by silica gel H (300–400 mesh) prepared thin-layer chromatography (p–TLC) with n–hexane–CH_2Cl_2–acetone (gradient, 30:1:0.1 to 5:1:0.1, v/v) then crystalized from PE–EtOAc mixed solvent to give **1** (12 mg). Fr. P_{II} (3 g) was performed on a silica gel CC (200–300 mesh), eluting with PE–Acetone (gradient, from 49:1 to 1:1, v/v) to yield four sub–fractions (sub-fraction P_{II}a–P_{II}d). Sub-fraction P_{II}c was isolated by silica gel H (300–400 mesh) prepared thin-layer chromatography (p–TLC) with cyclohexane–CH_2Cl_2–acetone (gradient, 25:1:0.1 to 5:1:0.1, v/v) then crystalized from PE–Acetone mixed solvent to give **2** (10 mg). Fr. P_{III} (5 g) was performed on a silica gel CC (200–300 mesh) eluting with PE–Acetone (gradient, from 29:1 to 1:1, v/v) to obtain four sub–fractions (sub-fraction P_{III}a–P_{III}d). Sub-fraction P_{III}a and P_{III}c were treated by repeated silica gel H (300–400 mesh) p–TLC with n–hexane–CH_2Cl_2–acetone (gradient, 30:2:0.1 to 4:1:0.1, v/v) and n–hexane–EtOAc–acetone (gradient, 35:1:0.1 to 5:1:0.1, v/v), then purified by Sephadex LH–20 CC with CH_2Cl_2–MeOH (3:1, v/v) to give **3** (9 mg) and **4** (10 mg), respectively. Fr. P_{IV} was subjected to a silica gel CC (200–300 mesh), isolated by PE–Acetone (from 35:1 to 1:1, v/v) to obtain three sub-fractions (sub-fraction P_{IV}a–P_{IV}c). Sub-fraction P_{IV}b and P_{IV}c were purified by repeated silica gel H p-TLC using n-hexane–EtOAc–ethyl ether (EE) (gradient, 30:1:0.5 to 10:1:0.5, v/v), then filtered by Sephadex LH–20 CC with CH_2Cl_2–MeOH (2:1, v/v) to give **5** (12 mg). Sub-fraction P_{V} was further performed on a silica gel CC (200–300 mesh) with PE–EtOAc (gradient, from 25:1 to 2:1, v/v), then crystallized from PE–EtOAc to yield **6** (60 mg).

The CH_2Cl_2 fraction (36 g) was solved in CH_2Cl_2–MeOH and mixed by silica gel (90 g) then evaporated to dryness. The mixture was performed on a silica gel (100–200 mesh) CC and separated by PE–Acetone (gradient, from 99:1 to 0:1, v/v) to give seven fractions (Fr. C_{I}–C_{VII}). Fr. C_{I} (21 g) was performed on a silica gel CC (200–300 mesh) eluting with PE–EtOAc (gradient, from 50:1 to 1:1, v/v) to give six sub-fractions (sub-fraction C_{I}A–C_{I}F). Sub-fraction C_{I}A was isolated by repeated silica gel H p-TLC with n-hexane–EtOAc–EE (gradient, 25:1:0.3 to 12:1:0.3, v/v) and crystallized from PE–EtOAc to obtain **5** (11 mg). Sub-fraction C_{I}B was separated on repeated silica gel H p-TLC with cyclohexane–EtOAc–EE (gradient, 28:1:0.5 to 10:1:0.5, v/v) and crystallized from PE–Acetone to obtain **6** (9 mg). Fr. C_{II} (19 g) was subjected to a silica gel CC (200–300 mesh) eluting with PE–Acetone (gradient, from 35:1 to 0:1, v/v) to give five sub-fractions (sub-fraction C_{II}A–C_{II}E). Sub-fraction C_{II}B was isolated by repeated silica gel H p-TLC with cyclohexane–Acetone–EE (gradient, 30:1:0.3 to 10:1:0.3, v/v), filtered by Sephadex LH-20 CC with CH_2Cl_2–MeOH mixture (gradient, from 7:3 to 1:2, v/v) and crystallized from CH_2Cl_2–MeOH to obtain **7** (16 mg). Sub-fraction C_{II}D was treated by repeated silica gel H p-TLC with PE–Acetone–EE (gradient, from 25:1:0.3 to 12:1:0.3, v/v), performed on Sephadex LH-20 CC with CH_2Cl_2–MeOH mixture (gradient, from 7:3 to 0:1, v/v), then crystallized from CH_2Cl_2–MeOH to give **8** (10 mg). Fr. C_{VII} (16 g) was subjected to a silica gel CC (200–300 mesh) eluting with CH_2Cl_2–MeOH (gradient, from 70:1 to 10:1, v/v) to give six sub-fractions (sub-fraction C_{VII}A–C_{VII}F). Sub-fraction C_{VII}D was further divided by repeated silica gel H p-TLC with CH_2Cl_2–Acetone–MeOH (gradient, from 45:1:0.5 to 5:1:0.5, v/v), filtered by Sephadex LH-20 CC with CH_2Cl_2–MeOH mixture (gradient, from 2:1 to 0:1, v/v) and crystallized from CH_2Cl_2–MeOH to obtain **9** (10 mg).

The EtOAc fraction (50 g) was solved in EtOAc–MeOH mixed solvent and absorbed by silica gel (90 g) then heated to dryness. The mixture was subjected to a silica gel (100–200 mesh) CC, eluted with CH_2Cl_2–MeOH (gradient, from 99:1 to 0:1, v/v) to give eight fractions (Fr. E_{I}–E_{VIII}). Fr. E_{I} (5 g) was performed on a silica gel CC (200–300 mesh) eluting with CH_2Cl_2–MeOH (gradient, from 50:1 to 10:1, v/v) to give six sub-fractions (sub-fraction E_{I}A–E_{I}F). Sub-fraction E_{I}A and E_{I}B were purified by Sephadex LH-20 CC with CH_2Cl_2–MeOH (gradient, from 1:1 to 0:1, v/v), then crystallized from MeOH to yield **10** (21 mg) and **11** (28 mg). Sub-fraction E_{I}F was further isolated by silica gel CC (200–300 mesh) with EtOAc–MeOH (gradient, from 35:1 to 15:1, v/v) to give **12** (19 mg). Fraction E_{II} (7 g) was performed on a silica gel CC (200–300 mesh) with CH_2Cl_2–MeOH as eluent (gradient, from 30:1 to 12:1, v/v) to obtain five sub-fractions (E_{II}A–E_{II}E). E_{II}C was subjected to a Sephadex LH-20 CC, eluting with CH_2Cl_2–MeOH (gradient, from 2:1 to 0:1, v/v), then crystallized MeOH to give **13** (9 mg). Fr. E_{III} (9 g) was subjected to a silica gel CC (200–300 mesh), eluted with CH_2Cl_2–MeOH (gradient, from 20:1 to 10:1, v/v) to give four sub-fractions (sub-fraction E_{III}A–E_{III}D). Sub-fraction E_{III}C and E_{III}D were decolorized by repeated Sephadex LH-20 CC (CH_2Cl_2–MeOH as elution solvent, from 1:1 to 0:1, v/v), crystallized from MeOH to obtain **14** (17 mg) and **15** (22 mg). Sub-fraction E_{V} (4 g) was treated by repeated silica gel CC (200–300 mesh) with CH_2Cl_2–MeOH (gradient, from 15:1 to 3:1, v/v) and Sephadex LH-20 CC with CH_2Cl_2–MeOH (gradient, from 1:2 to 0:1, v/v), then crystallized from MeOH to obtain **16** (18 mg). Sub-fraction E_{VII} (5 g) was isolated by repeated silica gel CC (200–300 mesh) with CH_2Cl_2–MeOH (gradient, from 10:1 to 2:1, v/v) and Sephadex LH-20 CC with CH_2Cl_2–MeOH (gradient, from 1:3 to 0:1, v/v), then crystallized from MeOH to give **17** (12 mg).

The structures of the obtained compounds were elucidated by the MS, ^{1}H NMR, ^{13}C NMR spectra and by comparison with those reported data in the related literature, which were identified as α-spinasterol (**1**) (Cheng, 2009; Ma, 2011), friedelin (**2**) (Jin et al., 2008), urs-12-ene (**3**) (Ma, 2011), olean-12-ene (**4**) (Ma, 2011), taraxerol (**5**) (Jin et al., 2008), oleanic acid (**6**) (Cheng, 2009), (+)-anhydrosecoisolariciresinol (**7**) (Thu et al., 2013), 18, 19-dihydroxy-5α, 10β-neo-cleroda-3, 13(14) dien-16, 15-butenolide (soulidiol) (**8**) (Wang et al., 1996), 18-O-β-ᴅ-glucopyranosyl-19-hydroxy-neo-cleroda-dien-butenolide (**9**) (soulidiol 18-O-β-D-glucoside) (Cui et al., 2003), *trans-p*-coumaryl alcohol (**10**) (Liu and Wang, 2014), *trans-p*-coumaryl aldehyde (**11**) (Liu and Wang, 2014), α-spinasterol-3-O-β-D-glucoside (**12**) (Jung et al., 2001), isorhamnetin (**13**) (Shen et al., 2012), emodin (**14**) (Ng et al., 2003), cosmosiin (**15**) (Li et al., 2015), (+)-epipinoresionol-4″-β-D-glucoside (**16**), (Sakamoto et al., 2008) and isoquercitrin (**17**) (Kim et al., 2014) (Fig. 1).

4. Chemotaxonomic significance

This present phytochemical study yielded seventeen chemical constituents from the alcohol extracts of the aerial part of S. nana, including two sterols (**1** and **12**), five triterpenoids (**2**–**6**), two lignanoids (**7** and **16**), two diterpenoids (**8** and **9**), two phenylpropanoids (**10** and **11**), three flavonoids (**13**, **15** and **17**) and one quinone (**14**). All of these compounds are obtained from *Sheareria nana* for the first time.

The isolated sterols are common in Compositae family, especially in Astereae, Heliantheae and Inuleae. Compounds **1** were reported from *Aster souliei* Franch (Astereae) (Cui et al., 2003; Cheng, 2009), *Aster ageratoides* Turcz. var. *oophyllus* Ling (Astereae) (Xi et al., 2003), *Aster scaber* (Thunb.) Nee (Astereae) (Jung et al., 2001), *Solidago canadensis* Lour (Astereae) (Ma, 2011), *Inula nervosa* Wall (Inuleae) (Yan et al., 2011), *Inala japonica* Thunb (Zhao, 2012) (Inuleae) and *Echinacea purpurea* (L.) Moench (Heliantheae) (Chen et al., 2013), while compound **12** was obtained from *A. scaber* (Astereae) (Jung et al., 2001), *Aster poliothamnus* Diels (Astereae) (He and Pan, 1992), *Conyza canadensis* (L.) Cronq. (Astereae) (Luo, 2005), *Solidago decurrens* Lour. (Astereae) (Xue, 2007) and