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GUAIANOLIDES FROM *SAUSSUREA INVOLUCRATA*

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Key Word Index—*Saussurea involucrata*; Compositae; guaianolides; 3α,8α-dihydroxy-11βH-,11,13-dihydrodehydrocostus lactone; involucratin.

Abstract—Seven guaianolides were isolated from *Saussurea involucrata*, four of them new. Their structures were elucidated by spectroscopic methods, some chemical transformations and X-ray diffraction.

INTRODUCTION

Saussurea involucrata (Kar. et Kir.) Sch.-Bip., a rare Chinese medicinal herb grown in Xinjiang province of China, has been used for the cure of rheumatic arthritis and gynopathy [1]. We previously reported sesquiterpenoids and flavonoids [2–5] from *S. involucrata*, while Bohlmann and Wang [6, 7] reported sesquiterpenoids. Here, we discuss the isolation and identification of some guaianolides. Two of these, (**1** and **2**) are already known and **3** was previously obtained from *Centaurea canariensis* [8], but this is the first report from *S. involucrata* [5]. The proof of structure **3** has been supplemented by ^{1}H NMR evidence in this paper. The other four compounds (**4**–**7**) are new guaianolides.

RESULTS AND DISCUSSION

The less polar lactone constituents of *S. involucrata* were identified as dehydrocostuslactone (**1**) and dihydrodehydrocostuslactone (**2**) [2, 3, 6]. The more polar lactones (**3**–**7**) were 8α-hydroxy, 11βH-11,13-dihydrodehydrocostuslactone; 11βH-11,13-dihydrodehydrocostuslactone 8-*O*-β-D-glucoside; 3α,8α-dihydroxy, 11βH-11,13-dihydrodehydrocostuslactone; 3α-hydroxy, 11βH-11,13-dihydrodehydrocostuslactone 8-*O*-β-D-glucoside and involucratin, respectively. The configurations and stereo chemistries of the five compounds were inferred from their IR, UV, NMR and mass spectra and by chemical transformations. The structure of **6** was confirmed by X-ray diffraction.

Compound **4** [5] was colourless needles, mp 219–221°, $C_{21}H_{30}O_8$, FDMS *m/z*: 410 $[M]^+$ ^{13}C NMR (after deducting aglycone signals: δ103.8, 77.1, 76.7, 73.9, 70.1, 61.2) showed that there was one glucose in this compound. Enzymatic hydrolysis afforded the aglycone which was identified as a guaianolide with a hydroxyl group on the basis of its IR, UV and ^{1}H NMR spectra. To determine the position of the hydroxyl, the aglycone was oxidized to the corresponding ketone. By comparison of the spectra of the aglycone with those of the ketone, the hydroxyl group was located at C-8. The IR, ^{1}H NMR spectra and mp of the aglycone were quite similar to that of a compound, which had been obtained previously from sabexpinnatin [9]. Therefore, compound **4** should be the glucoside of **3**. By comparing the ^{13}C NMR spectrum of **3** with that of **4**, the C-8 signal of **4** was shifted downfield to δ82.9 from 75.1, while the C-7 and C-9 signals were shifted upfield to 53.3 and 43.9 from 55.4 and 47.0, respectively. The glucose moiety was linked to the C-8 hydroxyl group of aglycone **3** and the anomeric proton signal of the glucose portion of **4** at δ4.32 (*d*, $J_{1,2} = 6.5$ Hz) showed that the glucosidic bond of **4** had the β-configuration.

Compound **5** was colourless needles, mp 157–158°, $C_{15}H_{20}O_4$. Its IR spectrum had bands at 3465, 3409 (OH), 1756 (α,β-unsaturated γ-lactone ring), 1637 (double bonds) cm^{-1}; and the mass spectrum had ions at *m/z*: 264 $[M]^+$, 246 $[M-H_2O]^+$ and 228 $[M-2H_2O]^+$. The ^{1}H NMR spectrum exhibited a doublet methyl signal at δ1.32, two pairs of exomethylene proton signals at 5.30, 5.22 (each *s*), 4.96, 4.82 (each *br*, *s*), and three protons on oxygenated carbons at δ4.61, 3.85 and 3.63. The mass spectrum of **5a** (acetylated derivative of **5**) had an ion at *m/z* 348 $[M]^+$ which confirmed the presence of two hydroxyl groups in **5** and therefore compound **5** was a guaianolide with two hydroxyl groups. By comparison of the ^{1}H NMR spectrum of **5a** with that of **5**, H-3 of **5a** was shifted downfield to δ5.60 from 4.61, and H-8 was shifted downfield to 4.95 from 3.63. There was no significant difference between the remaining proton signals of the two compounds, so the two hydroxyl groups could be located at C-3 and C-8. Compound **6**, mp 244–245°, $C_{21}H_{30}O_9$, FABMS *m/z*: 427 $[M+H]^+$, upon acid hydrolysis afforded glucose while enzymatic hydrolysis afforded the aglycone. The aglycone was identified as compound **5** on the basis of its IR, ^{1}H NMR, ^{13}C NMR and mass spectra. Therefore, compound **6** was the glucoside of **5**. The glucose moiety was found to be attached not to the oxygen function on C-3, but to that on C-8. By comparing the ^{13}C NMR spectrum of **5** with that of **6**, C-8 of **6** was shifted downfield to δ83.0 from 73.7, while the C-7 and C-9 were shifted upfield to 53.5 and 44.7 from 55.66 and 48.37, respectively. The structure was similar to that of 11βH-11,13-dihydrodesacylcynaropicrin 8-β-D-glucoside [10], but the H-3 signal showed that the hydroxyl groups had different configurations. This conclusion was verified by X-ray diffraction of **6** (Fig. 1).

Compound **7** is a novel sesquiterpene lactone alkaloid composed of dehydrocostus lactone and proline, the C-13

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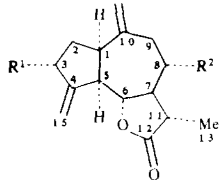
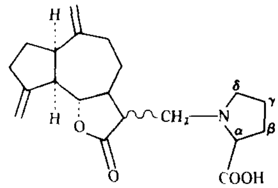


3 $R^1 = H, R^2 = OH$
4 $R^1 = H, R^2 = O$-β-Glc
5 $R^1 = OH, R^2 = OH$
5a $R^1 = R^2 = Ac$
6 $R^1 = OH, R^2 = O$-β-Glc



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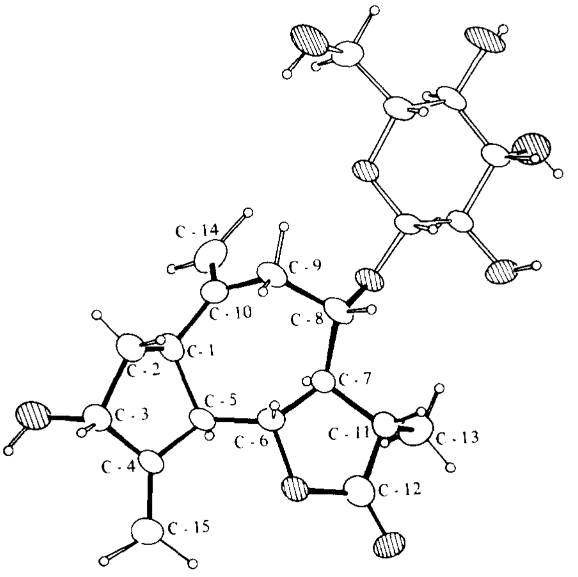

Fig. 1. The molecular structure of compound **6**.

of the dehydrocostus lactone being bonded to the nitrogen atom of proline.

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR: TMS as int. standard.

Plant material. Aerial parts of *S. involucrata* were collected from Xinjiang province of China. The material was identified and a voucher specimen is on deposit in the herbarium of Lanzhou University (No. 4721).

Extraction and isolation of lactones. The dried aerial parts of the plant were extracted with EtOH. After evapn of the solvent at red. pres., the concd aq. layer was extracted with petrol, Et_2O, EtOAc and *n*-BuOH, respectively. The petrol, EtOAc and *n*-BuOH extracts were chromatographed on a column of silica gel individually. Compounds **1**–**3** were obtained from the less polar solvents and compounds **4**–**7** from the more polar solvents.

8α-*Hydroxyl*-11βH-11,13-*dihydrodehydrocostuslactone* (**3**). Colourless needles (from Et_2O–petrol), mp 134–135°, $[\alpha]_D^{20}$ +15° (MeOH; *c* 0.17), hydroxamic acid test positive. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3450, 3007, 2900, 1760, 1645, 990, 900. UV $\lambda_{\max}^{\mathrm{MeOH}}$: 203 nm. MS *m/z*: 248 $[M]^+$, 230, 168, 156, 121, 107, 91, 79, 71, 67, 41 (100%). ^1H NMR (400 MHz, $CDCl_3$): δ5.21 and 5.05 (2H, each *d*, *J* = 1.8 Hz, H-15, 15′), 4.95 and 4.92 [2H, each *br s*, H-14, 14′], 3.92 (1H, *t*, *J* = 9.7 Hz, H-6), 3.74 (1H, *m*, *J* = 5, 2, 8.4, 9.3 Hz, H-8), 2.94 (1H, *m*, *J* = 5.9, 8.1, 9.8 Hz, H-1), 2.82 (1H, *t*, *J* = 9.8, 9.3 Hz, H-5), 2.72 (1H, *dd*, *J* = 5.2, 12.8 Hz, H-9), 2.58 (1H, *m*, *J* = 7.0, 10.8 Hz, H-11), 2.44 and 2.53 (2H, *m*, H-3, 3′), 2.20 (1H, *dd*, *J* = 8.1, 12.8 Hz, H-9′), 2.07 (1H, *q*, *J* = 9.7, 10.7 Hz, H-7), 1.85 (2H, *m*, H-2, 2′), 1.42 (3H, *d*, *J* = 7.1 Hz, H-13). ^{13}C NMR (20.13 MHz, $CDCl_3$): δ179.1 (*s*, C-12), 151.1 (*s*, C-4), 144.5 (*s*, C-10), 114.3 (*t*, C-15), 109.7 (*t*, C-14), 80.2 (*d*, C-6), 75.1 (*d*, C-8), 55.4 (*d*, C-7), 52.5 (*d*, C-5), 47.2 (*d*, C-1), 47.0 (*t*, C-9), 41.6 (*d*, C-11), 32.3 (*t*, C-2), 30.1 (*t*, C-3), 16.1 (*q*, C-13). (Found C: 73.03, H: 8.37, $C_{15}H_{20}O_3$, required C: 72.55, H: 8.12%). Acetylation of **3** yielded a solid, mp 138–142° (Et_2O–hexane). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3030, 2900, 1790, 1750, 1645. MS *m/z*: 290, 248, 231, 73. Oxidation of **3** (with 8 N CrO_3). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1762 (C=O of γ-lactone), 1713 (C=O of ketone). UV $\lambda_{\max}^{\mathrm{MeOH}}$: 203 nm, 208 nm (sh.). ^1H NMR (80 MHz, $CDCl_3$): δ5.25 (1H, *s*), 5.20 (1H, *s*), 5.05 (2H, *s*), 4.04 (1H, *t*, *J* = 9.6 Hz), 3.26 (2H, *s*), 2.95–3.44 (2H, *m*), 2.85 (1H, *br s*), 2.54 (2H, *t*, *J* = 7.2 Hz), 2.16 (1H, *s*), 1.89 (2H, *t*, *J* = 7.3 Hz), 1.25 (3H, *d*, *J* = 4.3 Hz).

11β*H*-11,13-*Dihydrodehydrocostuslactone* 8-O-β-D-*glucoside* (**4**). Colourless needles, mp 219–221°. Hydroxamic acid test positive and Molisch reaction positive. FDMS: 410, $C_{21}H_{30}O_8$, (Found C: 61.54, H: 7.45; required C: 61.42, H: 7.40%). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 3070, 2900, 1720, 1630, 910. EIMS *m/z*: 392, 248, 231, 132, 43 (100%). ^1H NMR [80 MHz, $(CD_3)_2SO$]: δ1.28 (3H, *d*, *J* = 7 Hz, Me), 4.32 (1H, *d*, *J* = 6.5 Hz, Glc C_1–H), 4.82–5.03 (4H, olefin). ^{13}C NMR [20.13 MHz, $(CD_3)_2SO$]: δ178.8 (C-12), 152.1 (C-4), 145.2 (C-10), 114.0 (C-15), 108.6 (C-14), 103.8 (Glc C-1), 82.9 (C-8), 79.2 (C-6), 77.1 (Glc C-3), 76.7 (Glc C-5), 73.9 (Glc C-2), 70.1 (Glc C-4), 61.2 (Glc C-6), 53.3 (C-7), 52.2 (C-5), 46.7 (C-1), 43.9 (C-9), 40.3 (C-11), 31.9 (C-2), 29.3 (C-3), 16.1 (C-13). Cellulase hydrolysis to **4** furnished **3**. After hydrolysis of **4** with dilute H_2SO_4, it was shown by paper chromatography that compound **4** contained one Glc residue. Acetylated compound **4**, colourless needles (from MeOH), mp 145–146°, HRMS $[M]^+$ at *m/z* 578.2381, $C_{29}H_{38}O_{12}$, other peaks: 331, 271, 231, 211, 169, 157, 145, 115, 109, 98, 43 (100%).

3α,8α-*dihydroxy*-11βH-11,13-*dihydrodehydrocostuslactone* (**5**). Colourless needles mp 157–158°. $C_{15}H_{20}O_4$, HRMS (found 264.1398 required 264.1362), $[\alpha]_D^{20}$ +64.2° (MeOH; *c* 0.112). Hydroxamic acid test positive. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3465, 3409, 3086, 2918, 1756, 1637, 1189, 1158, 1075, 982, 903. EIMS *m/z*: 264 (M^+, 100%), 246, 228, 200, 185, 173, 119, 105, 91, 79, 71, 55. ^1H NMR [80 MHz, $(CD_3)_2CO$]: δ3.01 (H-1), 2.10 and 1.90 (H-2, 2′), 4.61 (H-3), 2.85 (H-5), 3.85 (H-6), 2.20 (H-7), 3.63 (H-8), 2.85 and 2.20 (H-9, 9′), 2.63 (H-11), 1.32 (H-13), 4.96 and 4.82 (H-14, 14′), 5.30 and 5.22 (H-15, 15′). ^{13}C NMR [20.13 MHz, $(CD_3)CO$]: δ178.5 (C-12), 155.65 (C-4), 145.76 (C-10), 113.37 (C-15), 110.89 (C-14), 80.31 (C-6), 75.03 (C-3), 73.70 (C-8), 55.66 (C-7), 50.41 (C-5), 48.37 (C-9), 43.60 (C-1), 41.29 (C-2), 39.82 (C-11), 15.37 (Ć-13). Compound **5a** (Acetylate of **5**): ^1H NMR (80 MHz, $CDCl_3$): δ5.52 and 5.44 (2H, H-15, 15′), 5.30 and 5.07 (2H, H-14, 14′), 5.60 (1H, H-3), 4.95 (1H, H-8). MS *m/z*: 348 $[M]^+$, 305, 264, 228, 155, 91, 43.

3α-*Hydroxy*-11β*H*-11,13-*dihydrodehydrocostuslactone* 8-O-β-D-*glucoside* (**6**). Colourless needles, mp 244–245°. Hydroxamic acid test positive and Molisch reaction positive. FABMS 427 $[M+1]^+$, $C_{21}H_{30}O_9$, (Found C: 58.99, H: 7.20; required C: 59.15, H: 7.04). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3410, 3360, 2970, 2907, 1757, 1632, 1039. ^1H NMR (400 MHz, DMSO): δ 5.16 and 5.13 (2H, each *d*, H-15, 15′), 4.96 and 4.78 (2H, each *br s*, H-14, 14′), 4.45 (1H, *t*, *J* = 6.5 Hz, H-3), 3.83 (1H, *t*, *J* = 9.4 Hz, H-6), 3.61 (1H, *m*, *J* = 5, 10 Hz, H-8), 2.98 (1H, *m*, H-1), 2.94 (1H, *m*, *J* = 14, 5 Hz, H-9), 2.86 (1H, *q*, *J* = 10 Hz, H-5), 2.66 (1H, *m*, *J* = 10.4, 7 Hz, H-11), 2.15 (1H, *m*, *J* = 10 Hz, H-7), 2.20 (1H, *m*, *J* = 14, 10 Hz, H-9′), 2.0 (1H, *m*, *J* = 12, 7.4, 9 Hz, H-2), 1.64 (1H, *m*, *J* = 12, 7.4 Hz, H-2′), 1.29 (3H, *d*, *J* = 7 Hz, H-13), 4.31 (1H, *d*, *J* = 7.7 Hz, Glc H-1).

^{13}C NMR (20.13 MHz, DMSO): δ 178.8 (C-12), 155.04 (C-4), 144.8 (C-10), 114.14 (C-15), 110.7 (C-14), 103.6 (Glc C-4), 83.0 (C-8), 79.6 (C-6), 76.9 (C-3), 76.4 (Glc C-3), 73.6 (Glc C-5), 72.3 (Glc C-2), 70.0 (Glc C-4), 61.1 (Glc C-6), 53.5 (C-7), 49.9 (C-5), 44.7 (C-9), 43.1 (C-1), 40.1 (C-2), 39.1 (C-11), 16.06 (C-13). After hydrolysis of **6** by cellulase **5** was obtained and hydrolysis with dilute H_2SO_4 gave a Glc residue. Acetylation of **6** yielded crystals, EIMS: 636 $[M]^+$, 594, 331, 271, 169, 109.

Involucratin (**7**). Colourless needles, mp > 300°. Drangendorff reagent test: positive and Mayer reagent test: positive. IR ν_{max}^{KBr} cm^{-1}: 3403, 3000, 2951, 1761, 1634, 1456, 1402, 1374, 1323, 1213, 1171, 1013. EIMS *m/z*: 345 $[M]^+$, 300 (100%), 230, 201, 172, 150, 125, 105, 91, 70, 53. The constitution of some intense ion peaks in HRMS: $C_{19}H_{26}NO_2$ (Found 300.1958, required 300.1963), $C_{15}H_{18}O_2$ (Found 230.1298, required 230.1306), C_4H_8N (Found 70.0657, required 70.0656), ^{1}H NMR (400 MHz, DMSO): δ 5.02 and 4.97 (2H, each *br s*, H-15,15′), 4.89 and 4.83 (2H, each *br s*, H-14, 14′), 3.92 (1H, *t*, H-6), 3.2–3.4 (2H), 3.06 (2H, *m*), 2.89 (2H, *m*), 2.77 (1H, *t*), 2.59 (1H, *m*), 2.53 (1H, *d*), 2.45 (2H, *d*), 2.38 (2H, *m*), 2.02 (3H, *m*), 1.90 (1H, *m*), 1.80 (3H, *m*), 1.23 (1H, *m*), ^{13}C NMR (20.13 MHz, DMSO): δ 177.1 (C-12), 173.8 (proline COOH), 152.4 (C-4), 150.4 (C-10), 111.1 (C-15), 107.9 (C-14), 84.7 C-6), 66.7 (pro. Cα), 53.2 (C-13), 51.6 (C-5), 46.3 (C-1), 45.3 (C-7), 44.0 (pro. C δ), 40.6 (C-11), 36.6 (C-9), 32.1 (C-2), 31.4 (pro. Cβ), 29.5 (C-3), 28.6 (C-8), 23.5 (pro. Cγ).

X-Ray data of compound **6**. A crystal of dimensions $0.4 \times 0.3 \times 0.2$ mm was mounted on an Enraf–Nonius CAD4 diffractometer equipped with CuK$_\alpha$ radiation ($\lambda = 1.54184$ Å) and a graphite monochromater. Crystal data are: $C_{21}H_{30}O_9$, M_r 426.47, orthorhombic space group: $P2_12_12_1$: $a = 7.093$ (1) Å, $b = 13.365$ (1) Å, $c = 21.557$ (6) Å; $V = 2043.6$ Å^3; $Z = 4$; $Dc = 1.386$ Mgm^{-3}; F (000) = 912; μ(CuK$_\alpha$) = 0.86 mm^{-1}; T = 298 K. Data collection: $4° \leq 2\theta \leq 120°$; ω–2θ scan technique; 1781 total reflections; 1500 reflection with $I \geq 3\ \sigma$ (1) and were corrected for background, Lorentz and polarization effects.

The structure was solved by direct methods, using MULTAN-82 and Fourier techniques, refined by full-matrix least-squares. Nonhydrogen atoms were anisotropic. A difference Fourier synthesis upon convergence permitted location of 30 hydrogen atoms. In subsequent cycles of refinement the H atoms were assigned fixed isotropic thermal parameter. In the final cycle of refinement $R = 0.031$, $R_w = 0.032$ and the largest residual electron density was 0.22 eA^{-3}.

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