

Studies on the Constituents of Umbelliferae Plants. XVIII.¹⁾ Minor Constituents of Bupleuri Radix: Occurrence of Saikogenins, Polyhydroxysterols, a Trihydroxy C₁₈ Fatty Acid, a Lignan and a New Chromone

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Bupleuri radix, an important crude drug in the prescriptions of Chinese traditional medicine, was found to contain small amounts of previously unrecorded constituents, three saikogenins (1, 2 and 3), two polyhydroxysterols (4 and 5), one trihydroxy fatty acid (6a), one lignan (7a), and one simple new chromone, designated saikochromone A (8). These results represent a more complex profile of the chemical constituents of Bupleuri Radix, than was previously known.

Keywords Umbelliferae; *Bupleurum falcatum*; Bupleuri Radix; saikogenin; polyhydroxysterol; trihydroxy fatty acid; lignan; chromone

Bupleuri Radix (saiko), the dried roots of *Bupleurum falcatum* L., is one of the most frequently occurring crude drugs in the prescriptions of Chinese traditional medicine.²⁾ In contrast to its pharmacognostic importance and to much research performed, the chemical profile clarified to date was rather simple. The predominant constituents were the saikosaponins,³⁾ and so far the large numbers of chemical and pharmacological studies have been those regarding the saikosaponin derivatives. Occurrence of a coumarin,⁴⁾ several flavonoid derivatives,⁵⁾ and polyacetylenes,⁶⁾ together with such common compounds as fatty acids,^{3a)} sterols,^{3a)} streol glycoside,⁷⁾ and volatile carboxylic acids⁸⁾ have been reported, but it still seems too oversimplified to represent its chemical constituents as a whole. In view of the importance of Bupleuri Radix as one of the principal crude drugs, we reinvestigated its minor components.

The MeOH-CHCl₃ extract of Bupleuri Radix was partitioned with a mixture of CHCl₃-MeOH-H₂O (8:4:3).⁹⁾ This moved the bulk of the sugars and saponins into the aqueous layer. The organic layer was subjected to column chromatography. The hexane-CHCl₃ eluate afforded significant amounts of a crystalline saturated fatty acid mixture with a low melting point. Its proton nuclear magnetic resonance spectrum (¹H-NMR) revealed it to be composed of linear fatty acids. The mass spectrum (MS) showed the molecular ion peaks due to C₂₂, C₂₄, and C₂₃ acids as the major, and to C₂₅, C₂₆, and C₂₈ acids as the minor components. Chromatography of the more polar fraction gave compounds 7a and 8 from the CHCl₃ eluates, compounds 1 and 2 from the 2% MeOH-CHCl₃ eluates, and compounds 3, 4, 5 and 6b from the 5% MeOH-CHCl₃ eluates.

Compounds 1, 2 and 3 were found to be saikogenins G, F and D, respectively. They were identified by comparisons of their spectral properties,^{7,10,11)} with those reported in the literatures. The dihydrofuran rings of saikogenins G and F are labile to acidic conditions, and give conjugated dienes, or ethers derived from the hydroxylic solvents used in the reaction.^{3a)} During the extraction and separation process, none of such acidic conditions were employed, and saikogenin D (3) was assumed to be the constituent originally present in Bupleuri Radix. Saikogenin E,¹²⁾ another known genuine sapogenin having a dihydrofuran ring, was not found. Although there are numerous reports regarding saikosaponins,³⁾ curiously enough this is the first

confirmation of the saikogenin itself in Bupleuri Radix.

Compounds 4 and 5 were obtained in a crystalline 2:1 mixture which was resistant to separation. The ¹H-NMR signals, due to the four secondary and two quaternary methyl groups (Experimental) of the major component 4, were distinguishable by subtracting those of 5 (*vide infra*). These, together with its MS (M⁺, *m/z* 330), suggested it to be a trihydroxyergostane-type steroid with two double bonds, one of them a 22*E*-double bond.¹³⁾ The chemical shifts (in pyridine-*d*₅), corresponding to H-19 (δ 1.55), H-3α (4.85, m) and H-4β (3.05, dd, *J*=11.5, 13.0 Hz), were intensely down-field shifted by the pyridine-induced deshielding effect,¹⁴⁾ as compared with those taken in CDCl₃-CD₃OD (1:1). These signals and those of H-18 (δ 0.68)¹⁵⁾ and a deshielded olefinic proton (5.75) indicated it to be 24ξ-methylcholesta-7,22*E*-diene-3β,5α,6β-triol. Its 24*S* and 24*R* isomers have been obtained from a marine sponge.¹⁶⁾ The ¹H-NMR spectrum of 4 was identical with that of the sponge sterol which was assigned as having a 24*R* configuration, though in our opinion this assignment is unreliable.¹⁷⁾ Treatment of the mixture of 4 and 5 with H₂SO₄ in MeOH-H₂O caused the decomposition of 4 and the minor component 5 was isolated. The ¹H-NMR of 5 showed the same signals with those of 24-methylcholestan-3β,5α,6β,25 tetrol,¹⁸⁾ except that 5 has a stigmastane-type side chain.¹³⁾ 24ξ-Ethylcholest-22*E*-ene-3β,5α,6β-triol has previously been found in a crude drug *Fritillariae bulbosae*.¹⁹⁾

Compound 6a, isolated as the methyl ester (6b), has previously been obtained from the Umbelliferae crude drugs *Angelicae dahuricae* Radix and *Angelicae* Radix.²⁰⁾ It was a trihydroxy C₁₈ fatty acid having one *trans*-disubstituted double bond (δ 5.81 and 5.72, each dd, *J*=16.0, 5.9 Hz) and formed an acetonide 6c. The ¹H-NMR of 6b indicated that the double bond was linked to a hydroxymethine (δ 4.14, dt) and a glycol group (δ 3.95, dd; 3.47, m). The MS of the tetramethylsilyl (TMS) ether of 6b showed the characteristic cleavage of the glycol group,²¹⁾ giving ions at *m/z* 173 and 387 and indicated 6a to be 9,12,13-trihydroxy-10*E*-octadecenoic acid. The ¹H-NMR spectra of 6b and 6c coincided with those of the authentic 15,16-dihydrofulgidic acid (9*S**,12*S**,13*S**)²²⁾ and its acetonide, respectively. The benzoate 6d derived from 6c showed the positive cotton effect (227 nm, Δε, +1.33) so that the absolute configuration of 6a was shown to be 6*S*,12*S*,13*S*.

Examination of the heteronuclear multiple bond cor-

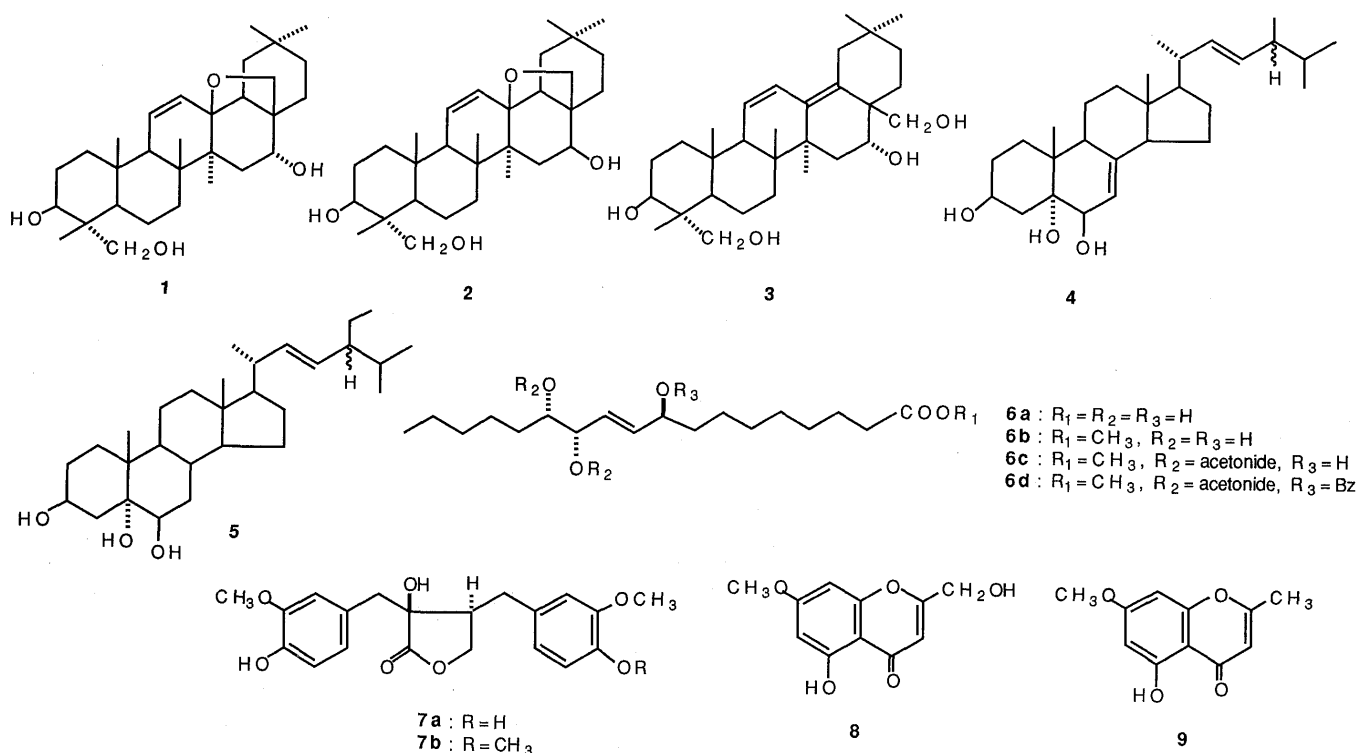


Chart 1

relation spectroscopy (HMBC) spectrum of **7a** led to the structure of a known lignan nortrachelogenin,²³⁾ a cyclic adenosine monophosphate (AMP) phosphodiesterase inhibitor.²⁴⁾ Its methyl derivative trachelogenin (**7b**) is known as a potent Ca^{2+} antagonist²⁵⁾ having a prolonged anti-hypertensive effect.

The 1H -NMR data of **8** suggested it to be a simple chromone. It showed signals of three olefinic protons (δ 6.48, 6.59 and 6.78), one methoxy (δ 3.73), and one hydroxymethyl (δ 4.75) group. The infrared (IR) band at 1660 cm^{-1} and the ^{13}C -NMR signal at δ 183.0 indicated the existence of one conjugated carbonyl group. Its heteronuclear multiple quantum coherence (HMQC) and HMBC spectra showed the presence of a 6-hydroxy-4-methoxy-1,2-disubstituted benzene ring, and a β -oxygenated- β -hydroxymethyl conjugated ketone moiety which was fused to the benzene ring. The α -proton (δ 6.78) of the enone moiety was correlated with one of a quarternary carbon (C-4a, δ 106.1) of the benzene ring, which is shifted to high-field by the effect of the neighboring oxygen atoms. The β -oxygen atom of the enone moiety was linked to a quarternary carbon (C-8a, δ 158.2) of the benzene ring, thus causing similarly a significant shielding effect to the adjacent two carbons at δ 92.8 (C-8) and 106.1. Comparison of the 1H -NMR data with that of the known compound eugenin (**9**)²⁶⁾ revealed their close analogy except for the C-2 substituents. Compound **8** was thus shown to be 5-hydroxy-2-hydroxymethyl-7-methoxy-4*H*-benzopyran-4-one. This simple chromone had not been reported previously. In view of the possible existence of analogous chromone derivatives in Bupleuri Radix, we propose the trivial name saikochromone A for **8**.

Experimental²⁷⁾

Fractionation of Bupleuri Radix Extract The pulverized material

(8.5 kg) was extracted with hexane and $CHCl_3$ -MeOH (1:1). The $CHCl_3$ -MeOH extract (1470 g) was partitioned with a mixture of $CHCl_3$ -MeOH- H_2O (8:4:3)⁹⁾ and separated to upper (935 g) and lower (376 g) extracts. The lower extract (300 g) was charged on a column of silica gel (1.5 kg) and eluted (2 l/fr) with a mixture of hexane- $CHCl_3$ (1:1, frs. 1-7), then with $CHCl_3$ (frs. 8-15), 2% MeOH in $CHCl_3$ (frs. 16-23), 5% MeOH in $CHCl_3$ (frs. 24-29), 20% MeOH in $CHCl_3$ (frs. 30-33), and the lower layer of a mixture of $CHCl_3$ -MeOH- H_2O (6.5:3:1, frs. 34-41).

Saikogenin G (1) and Saikogenin F (2) A portion (9.3 g) of combined frs. 17-24 (73.5 g) was passed through a column of alumina with 5% MeOH in $CHCl_3$ to remove the acidic materials. The combined eluates (1.33 g) were chromatographed with 5% MeOH in $CHCl_3$ giving a mixture (408 mg) containing **1** and **2**. It was chromatographed with 2% MeOH in $CHCl_3$ giving mixtures containing **1** (145 mg) and **2** (155 mg). Both mixtures were purified by chromatography with ethyl acetate-hexane (2:1) giving **1** (62 mg) and **2** (28.6 mg). **1**, mp 232-237°C from acetone-hexane, $[\alpha]_D^{25} + 75^\circ$ ($c=0.48$, EtOH). 1H -NMR ($CDCl_3$) δ : 0.84, 0.92, 0.94, 0.96, 1.04, 1.26 (each 3H, s), 3.17, 3.45 (each 1H, d, $J=7.3$ Hz), 3.38, 4.02 (each 1H, d, $J=10.5$ Hz), 3.63 (1H, m), 3.97 (1H, br d, $J=5.0$ Hz), 5.42 (1H, dd, $J=10.5, 3.0$ Hz), 5.90 (1H, br d, $J=10.5$ Hz). ^{13}C -NMR (pyridine- d_5): identical with those in reference 7. MS m/z : 472 (M^+), 457, 454, 441, 423, 405, 399, 349, 347, 320, 306, 302, 273, 271. **2**, mp 235-237°C from acetone-hexane, $[\alpha]_D^{25} + 93^\circ$ ($c=0.82$, EtOH). 1H -NMR ($CDCl_3$) δ : 0.84, 0.91, 0.97, 1.00, 1.08 (each 3H, s), 3.11, 3.91 (each 1H, d, $J=7.3$ Hz), 3.39, 3.40 (each 1H, br d, $J=10.5$ Hz), 3.55-3.70 (1H, m), 4.18 (1H, dd, $J=10.0, 6.0$ Hz), 5.42 (1H, dd, $J=10.0, 3.0$ Hz), 5.88 (1H, d, $J=10.0$ Hz). ^{13}C -NMR (pyridine- d_5): identical with those in reference 7. MS m/z : 472 (M^+), 457, 454, 441, 424, 423, 405, 399, 347, 306, 187.

Saikogenin D (3), 24 ξ -Methylcholesta-7,22*E*-diene-3 β ,5 α ,6 β -triol (4), 24 ξ -Ethylcholesta-22*E*-ene-3 β ,5 α ,6 β -triol (5) and 9*S*,12*S*,13*S*-Trihydroxy-10*E*-octadecenoic Acid Methyl Ester (6b) Frs. 26-30 (20.2 g) was chromatographed with 5% and 10% MeOH in $CHCl_3$ giving subfractions 1 (10.8 g), 2 (7.85 g) and 3 (2.43 g). A portion (690 mg) of the subfraction 2 was treated with diazomethane solution in Et_2O , giving a mixture containing carboxylic acid methyl esters. It was chromatographed with acetone- $CHCl_3$ (1:3) giving **6b** (11.2 mg). Subfraction 3 (2.43 g) was chromatographed over a column of alumina with $CHCl_3$ then 5% MeOH in $CHCl_3$ giving **3** (44.7 mg) and a mixture (80 mg) containing **3** and very small amounts of polyhydroxysterols **4** and **5**. The mixture gave, by dissolving in small amounts of MeOH, a crystalline mixture of **4** and **5** (2:1, 2 mg) which was filtered off. The mother liquor was evaporated and

the residue was dissolved in a mixture of 20% H₂SO₄ (1 ml) and MeOH (3 ml) and kept at room temperature for 3 h. The mixture was diluted with Et₂O and H₂O, and the Et₂O layer was washed with H₂O, saturated NaCl solution, then the solvent was evaporated off. The residue was chromatographed with 10% MeOH in CHCl₃ giving 30 mg of **3** and 2 mg of **5**.

Saikogenin D (3): mp 253–259 °C from ethyl acetate–hexane, $[\alpha]_D^{42}$ ($c=0.95$, EtOH). ¹H-NMR (CDCl₃) δ : 0.71, 0.84, 0.85, 0.90, 0.93, 0.97, 1.22, 1.23 (each 3H, s), 3.40 (2H, m), 3.6–3.8 (4H, m), 4.05 (1H, t, $J=3.7$ Hz), 5.57 (1H, d, $J=10.5$ Hz), 6.44 (1H, dd, $J=10.5$, 3.3 Hz). ¹³C-NMR (pyridine-*d*₅): identical with those in reference 7. MS m/z : 472 (M^+), 441, 423, 405, 347, 187.

24-Methylcholesta-7,22E-diene-3 β ,5 α ,6 β -triol (4): Data were derived from those of the mixture of **4** and **5** by subtracting the data of **5**. ¹H-NMR (pyridine-*d*₅) δ : 0.68 (3H, s), 0.87, 0.88 (each 3H, d, $J=6.5$ Hz), 0.97 (3H, d, $J=7.0$ Hz), 1.08 (3H, d, $J=6.2$ Hz), 1.55 (3H, s), 3.05 (1H, dd, $J=11.5$, 13.0 Hz), 4.34 (1H, m), 4.85 (1H, m), 5.23 (2H, m), 5.75 (1H, m). MS m/z : 430 (M^+), 412, 394, 379, 269, 251. High-resolution MS [Found (Calcd)] m/z : C₂₈H₄₄O₂ ($M^+ - H_2O$), 412.33313 (412.33403).

24-Ethylcholesta-22E-ene-3 β ,5 α ,6 β -triol (5): mp 255–258 °C from acetone–hexane, $[\alpha]_D^{20}$ ($c=0.064$, EtOH). ¹H-NMR (pyridine-*d*₅) δ : 0.78 (3H, s), 0.89 (3H, t, $J=7.3$ Hz), 0.86, 0.91 (each 3H, d, $J=6.2$ Hz), 1.10 (3H, d, $J=6.5$ Hz), 1.68 (3H, s), 2.98 (1H, dd, $J=11.3$, 12.3 Hz), 4.17 (1H, br s), 4.8–5.0 (1H, m), 5.07, 5.20 (each 1H, dd, $J=15.0$, 8.8 Hz). MS m/z : 446 (M^+), 428, 410, 367, 349, 334, 316, 305, 287, 271, 253. High-resolution MS, [Found (Calcd)] m/z : C₂₉H₅₀O₃ (M^+), 446.3730 (446.3759).

Compound 6b: mp 93–94 °C, $[\alpha]_D^{20} -11^\circ$ ($c=0.60$, CHCl₃). ¹H-NMR (CDCl₃) δ : 0.89 (3H, t, $J=7.0$ Hz), 2.30 (2H, t, $J=7.5$ Hz), 3.47 (1H, m), 3.67 (3H, s), 3.95 (1H, dd, $J=6.2$, 5.9 Hz), 4.14 (1H, dt, $J=6.2$, 5.9 Hz), 5.72, 5.81 (each 1H, dd, $J=16.0$, 5.9 Hz). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3550, 3300, 1725, 975, 860. MS (as TMS ether) m/z : 545 ($M^+ - Me$), 460 ($M^+ - \text{hexanal}$), 387, 173. Anal. Calcd for C₁₉H₃₄O₅: C, 66.24; H, 10.53. Found: C, 66.09; H, 10.76.

Acetonide (6c) A solution of **6b** (4 mg) in 2,2-dimethoxypropane (2 ml) was treated with 1 mg of *p*-TsOH at room temperature for 3 h. After the addition of 2 ml of saturated NaHCO₃ solution, the mixture was stirred for 15 min. The organic layer was washed with H₂O, saturated NaCl solution, and dried over Na₂SO₄. Chromatography of the evaporation residue with ethyl acetate–hexane gave **6c** (2.2 mg) as an oil. ¹H-NMR (CDCl₃) δ : 0.89 (3H, t, $J=7.0$ Hz), 2.30 (2H, t, $J=7.3$ Hz), 3.67 (3H, s), 3.67 (1H, m), 4.00 (1H, t, $J=7.9$ Hz), 4.15 (1H, m), 5.65 (1H, ddd, $J=15.4$, 7.5, 1.0 Hz), 5.84 (1H, ddd, $J=15.4$, 5.9, 1.0 Hz). Compound **6c** (1 mg) was dissolved in a mixture of pyridine (2 drops) and benzoylchloride (1 drop) and was kept at 50 °C for 6 h. The mixture was diluted with Et₂O and washed with 2N HCl, H₂O, saturated NaCl solution and the solvent was evaporated. The residue was purified over a column of silica gel, giving monobenzoate of **6d** (0.2 mg) as an oil. CD spectrum, see the Text.

Nortrachelogenin (7a) and Saikochromone A (8) Frs. 10–16 were combined (14.82 g) and separated into subfractions 1 to 25 with acetone–hexane (1:2). Repeated chromatography of the combined subfractions 6 and 7 (2.6 g) gave **8** (76.6 mg), mp 185–192 °C from acetone–CHCl₃ (15:85). ¹H-NMR (pyridine-*d*₅) δ : 3.73 (3H, d, $J=1.0$ Hz), 4.75 (2H, s), 6.48 (1H, d, $J=2.0$ Hz), 6.59 (1H, d, $J=2.0$ Hz), 6.78 (1H, br s), 7.84 (1H, br). ¹H-NMR (in CDCl₃) δ : 3.86 (3H, s), 4.57 (2H, br d), $J=5.5$ Hz), 6.33 (1H, t, $J=1.0$ Hz), 6.36, 6.37 (each 1H, d, $J=2.0$ Hz). ¹³C-NMR (pyridine-*d*₅) δ : 55.9 (q), 60.2 (t), 92.8, 98.4 (each d), 106.1 (s), 106.4 (d), 158.2, 162.7, 165.8, 171.6, 183.0 (each s). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3300, 1660, 1635, 1580, 1520. MS m/z : 222 (M^+), 193. High-resolution MS [Found (Calcd)] m/z : C₁₁H₁₀O₅ (M^+), 222.05121 (222.05281). Chromatography of the subfractions 10–13 (960 mg) with acetone–CHCl₃ (1:9) gave an unknown compound (16.5 mg) and a mixture containing **7a** (155 mg). The mixture was purified by chromatography with Et₂O–CHCl₃ (1:3) giving **7a** (124.3 mg) as a colorless oil, $[\alpha]_D^{20} -40^\circ$ ($c=1.98$, EtOH). ¹H-NMR (pyridine-*d*₅) δ : 2.85 (1H, m), 2.97 (1H, dd, $J=13.5$, 10.0 Hz), 3.30 (1H, dd, $J=13.5$, 4.5 Hz), 3.38 (1H, d, $J=13.5$ Hz), 3.69 (1H, d, $J=13.5$ Hz), 3.775, 3.782 (each 3H, s), 4.17, 4.31 (each 1H, t, $J=8.5$ Hz), 6.90 (1H, dd, $J=8.0$, 2.0 Hz), 6.97 (1H, d, $J=2.0$ Hz), 7.12 (1H, dd, $J=8.0$, 2.0 Hz), 7.17 (1H, d, $J=2.0$ Hz), 7.21, 7.24 (each 1H, d, $J=8.0$ Hz, overlapped by solvent signals). ¹³C-NMR (pyridine-*d*₅) δ : 31.7, 41.7 (each t), 44.2 (d), 55.7, 56.0 (each q), 70.9 (t), 76.7 (s), 113.3, 114.9, 116.5, 116.7, 122.2, 124.0 (each d), 127.5, 130.8, 146.8, 147.4, 148.6, 148.8, 179.4 (each

s). MS m/z : 374 (M^+), 137. IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 3400, 1760, 1605, 1510. High-resolution MS [Found (Calcd)] m/z : C₂₀H₂₂O₇ (M^+), 374.13270 (374.13650).

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