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Solubilizing Properties of Glycyrrhizin and Its Derivatives: Solubilization of Saikosaponin-a, the Saponin of Bupleuri Radix

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Licorice root is often co-prescribed with Bupleuri Radix (Bupleurum root) for decoctions used in oriental traditional medicine. It was found that the water solubility of saikosaponin-a, the active principle of Bupleurum root, was increased in the presence of the water extract or the saponin fraction of licorice root and this solubilizing effect was due to glycyrrhizin, the major active saponin of this plant drug. A solubilizing effect on saikosaponin-a was also observed with the 30-β-glucoside ester and 30-β-glucuronide ester of glycyrrhizin. The 30-β-glucoside ester improved the solubilizing property of glycyrrhizin. Aqueous solutions of the 30-β-glucoside ester and the 30-β-glucuronide ester solubilized *dl*-α-tocopherol and oleanolic acid, both of which are almost insoluble in water.

Keywords——licorice root; Bupleurum root; glycyrrhizin; saikosaponin-a; solubilizing effect; glycyrrhizin 30-glycosyl ester; saponin; α-tocopherol; oleanolic acid; oriental traditional medicine

Bupleuri Radix (Bupleurum root), the root of *Bupleurum falcatum* L. and related species, is an important oriental traditional medicine. The active principles of this crude drug, saikosaponin-a (**1**) and -d (**2**), are known to be sparingly soluble in water. Ginseng is sometimes co-prescribed with Bupleurum root in kampo-decoctions (formulae in oriental traditional medicine). In our serial studies on the solubilizing effect of natural oligoglycosides, it has been revealed[1,2] that ginsenoside Ro (**3**), the acidic oleanane saponin of ginseng, remarkably increases the water solubility of **1** and **2**, while no solubilizing effect on **1** and **2** was observed with the neutral dammarane saponins of ginseng. However, it has been found that this solubilizing effect of **3** is significantly potentiated in the presence of the neutral dammarane saponins, and the enhancement of the water solubility of **1** and **2** with ginseng extract or its saponin fraction can be explained in terms of the cooperative effect of **3** with the dammarane saponins. The important role of the glucuronide moiety of **3** in this solubilizing effect has also been elucidated. No solubilizing effect on **1** and **2** has been observed with the neutral saponins, bisdesmosides from *Sapindus* spp.[3-5] and cyclic bisdesmosides[6,7] from *Bolbostemma paniculatum*, which greatly increase the water solubility of other substances.

Glycyrrhizae Radix (licorice root), a well-known plant drug, is more often co-prescribed with Bupleurum root than ginseng. The present paper deals with the solubilizing effect of saponins of licorice root on **1**.

It was found that a water extract of licorice root increased the water solubility of **1**. The water extract was chromatographed on highly porous polymer and eluted with water, 80% methanol, methanol and acetone, successively. The eluate with 80% methanol showed the strong solubilizing effect on **1**. This effective fraction mainly consisted of the well-known major saponin, glycyrrhizin (**4**) and other minor saponins, being named the saponin fraction (S-fr). The contents of **4** in the water extract and S-fr were determined: 18% in the former and

39% in the latter. Based on these analytical results, the solubilizing effects of the water extract and S-fr were compared with that of **4** (Fig. 1); a 0.036% aqueous solution of **4** dissolved a similar amount of **1** to that with 0.2% solution of the water extract, and 0.039 and 0.079% solutions of **4** exhibited similar solubilizing effects on **1** to those of 0.1 and 0.2% solutions of S-fr, respectively. This indicates that the solubilizing effects of the water extract and S-fr on **1** are due to **4**. It is noteworthy that as in the case of **3**, which shows a strong solubilizing effect on **1**, **4** is also a glucuronide saponin (*vide supra*).

Very recently, Kitagawa *et al.*[8] isolated the 30-β-glucoside ester (**5**) of **4** from licorice roots as a minor saponin. This bisdesmoside (**5**) and the 30-β-glucuronide ester (**6**) were prepared from **4** through a dimethyl ester (**7**). It was found that both **5** and **6** also increased the water solubility of **1** when used at somewhat higher concentrations than **4** (Figs. 1 and 2).

It was observed that in the case of **4**, the solubilizing effect was decreased at relatively high concentration. This would be due to the increase of viscosity of the solution (gelation) with increasing concentration. Such a phenomenon was not observed for the bisdesmosidic saponins, **5** and **6**. Further, it was found that this decrease of the solubilizing effect of **4** was

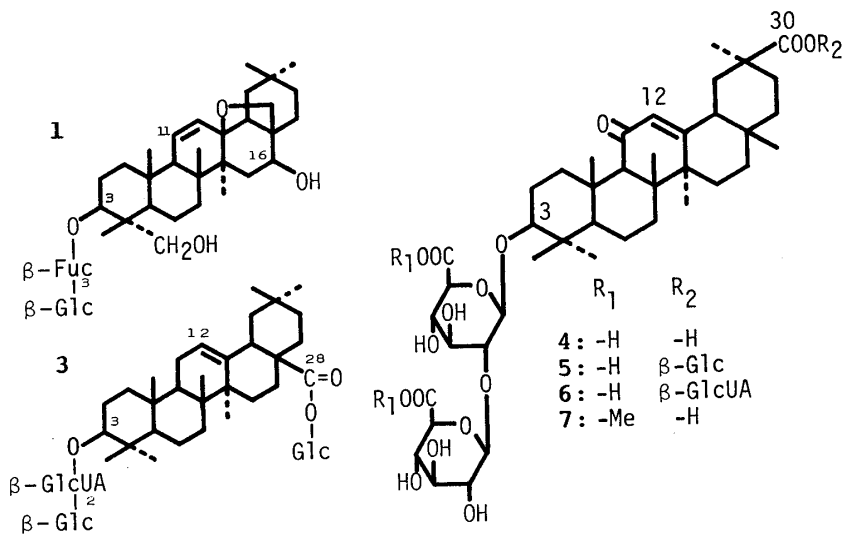

Chart 1

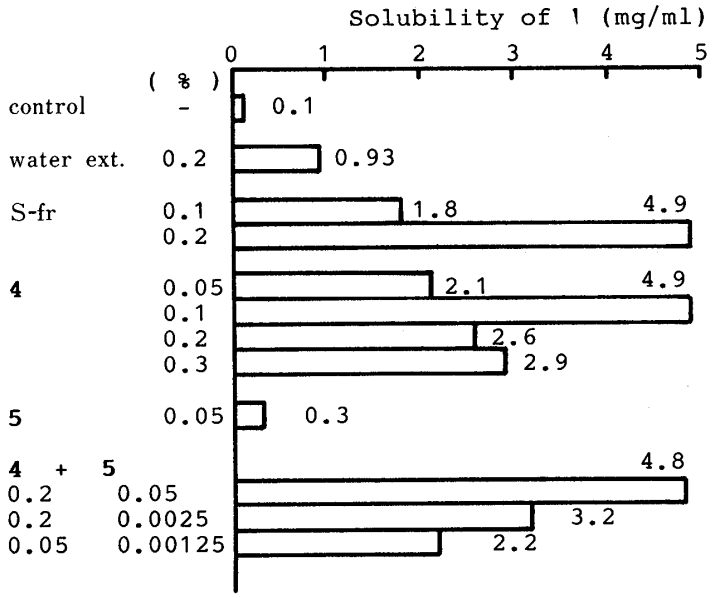

Fig. 1. Solubilizing Effects on Saikosaponin-a (**1**) (37 °C, 24 h, H_2O)

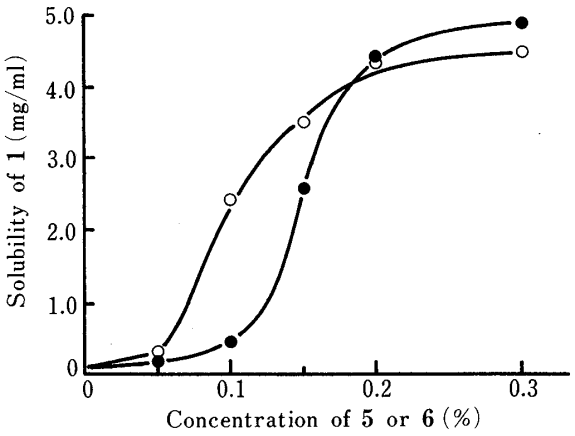

Fig. 2. Solubilizing Effects on Saikosaponin-a (**1**) (37 °C, 24 h, H_2O)
○, **5**; ●, **6**.

modified in the presence of **5** by suppression of the gelation; a 0.1% aqueous solution of **4** dissolved 4.9 mg/ml of **1** at 37 °C, while a 0.2% aqueous solution dissolved only 2.6 mg/ml of **1**. As shown in Fig. 1, a 0.05% aqueous solution of **5** showed no solubilizing effect on **1**, while a 0.2% solution of **4** containing 0.05% of **5** dissolved 4.8 mg/ml of **1** (see Fig. 1).

Compounds **5** and **6** also increased the water solubilities of *dl*-α-tocopherol (**8**) and oleanolic acid (**9**), both of which are almost insoluble in water; in 0.1% aqueous solutions of **5** and **6**, **8** was dissolved at concentrations of 78 and 25 μg/ml, respectively, at 37 °C. Compound **8** was emulsified in an aqueous solution of **4**. Aqueous 0.2% solutions of **5** and **6** dissolved 57 and 63 μg/ml of **9**, respectively. No solubilizing effect on glycyrrhetinic acid, the aglycone of **4**, or progesterone was observed with **5** and **6**.

The saponin **4** is known as a sweetener, while the esters **5**, **6** and **7** are tasteless.

Experimental

Materials——**4**: The dipotassium salt of **4** (supplied by Maruzen Kasei Co., Ltd., Onomichi, Hiroshima-ken) was treated with Amberlite MB-3 or Dowex 50W-X8 and the resulting free saponin was further purified by column chromatography on silica gel [solvent: $CHCl_3$–MeOH–H_2O (6:4:1, homogeneous)] followed by high-performance liquid chromatography (HPLC) on TSKgel ODS-120T (Tosoh Co., Ltd., 21.5 mm i.d. × 300 mm); mobile phase, 5% AcOH–MeOH–2-PrOH (30:65:5); flow rate, 6 ml/min; detection, ultraviolet (UV) 254 nm and refractive index (R.I.). **1** was obtained from Bupleurum root according to our previous paper.[9] Commercial *dl*-α-tocopherol (Tokyo Kasei Kogyo Co., Ltd., lot AOO1) and progesterone (Sigma Co., Ltd., lot 32F-0102) were used. Glycyrrhetinic acid was supplied by Maruzen Kasei Co., Ltd., and **9** was obtained in our previous paper.[10]

Extraction of Licorice Roots——Commercial licorice roots (Kojima Kampo Co., Ltd., Osaka, lot 202006, 200 g) was extracted with hot water (750 ml) four times. The combined solutions were concentrated to dryness to give the water extract (63 g). The water extract (20 g) was chromatographed on Diaion HP-20 and eluted with H_2O, 80% MeOH, MeOH and acetone. The solubilizing effect on **1** was observed not with the water, MeOH and acetone eluates, but with the 80% MeOH eluate (S-fr, 8.4 g).

Quantitative Analysis of 4——Contents of **4** in the water extract and S-fr were determined by dual-wavelength thin layer chromatogram (TLC) densitometry on a Shimadzu CS-930 TLC scanner, equipped with a Shimadzu DR-2 Data-recorder; silica gel plate, Kieselgel 60 F_{254} (Merck, Art. 11798), 20 × 20 cm; solvent, [AcOH]–[$CHCl_3$–MeOH–H_2O (6:4:1, homogeneous)] (5:95); scanning parameter, λ_s 264 nm, λ_r 370 nm. In every experiment, standard solutions of **4** were spotted on each plate to obtain the calibration curve.

Determination of Solubility of 1——The solubilizing test was carried out according to the method of the previous paper.[1]

Determination of Solubility of 8——Water (22.1 ml) was added to 22.1 ml of a solution of **8** (97.3 mg) in EtOH (50 ml), and the solution was diluted with 50% EtOH to 1000 ml to give a 0.1 mM solution of **8** in 50% EtOH. Using this standard solution, calibration plots based on transmittance at UV 292 nm were obtained. It was confirmed that the calibration curve is linear in the range of concentrations from 0.01 to 0.1 mM.

The extract, a fraction or a solubilizing agent was added to a solution of **8** (15 mg) in EtOH (1 ml), and the solution was concentrated to dryness by blowing N_2 gas over it. After complete drying *in vacuo* overnight, the residue was taken up in H_2O (3 ml) and the mixture was sonicated and then incubated at 37 °C for 24 h. The mixture was

filtered and 2 ml of the filtrate was diluted with EtOH (2 ml), for determination of the UV transmittance at 292 nm. The presence of **4**, **5** or **6** did not disturb the analysis of **8**.

Determination of Solubility of 9——Compound **5** or **6** was added to a solution of **9** (1.5 mg) in EtOH (1 ml), and the solution was concentrated to complete dryness. The residue was taken up in H_2O (1.5 ml), sonicated and then incubated at 37 °C for 24 h. The mixture was filtered, 0.8 ml of the filtrate was diluted with EtOH (0.4 ml), and the solution was analyzed by dual-wavelength TLC densitometry on silica gel (Kieselgel $60F_{254}$, 20 × 20 cm); solvent, C_6H_6–acetone (4 : 1); visualized by spraying 10% H_2SO_4 followed by heating 110 °C for 10 min.[3] In every experiment, standard solutions of **9** were spotted on the same plate to make a calibration curve. Scanning parameters: λ_s 393 nm, λ_r 700 nm.

Preparation of the Dimethyl Ester (7) from 4——A solution of **4** (517 mg) in 2% methanolic HCl (35 ml) was stirred at room temperature for 2 h. The solution was treated with Ag_2CO_3 and the precipitate was removed by filtration. The filtrate was concentrated to dryness and the residue was chromatographed on silica gel [solvent: $CHCl_3$–MeOH–H_2O (40 : 15 : 2, homogeneous)] to give **7** in a yield of 75%. **7**: A white powder. $[\alpha]_D^{19}$ +55.5° (c = 1.03, MeOH). Proton nuclear magnetic resonance (^{1}H-NMR) (C_5D_5N) δ: 3.71, 3.84 (3H s each, –OCH_3). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum in C_5D_5N, carbon signals due to carboxyl groups of the glucuronide moieties were shifted to δ 170.2 (2C) in **7** from δ 172.1 (1C) and 172.5 (1C) in **4**, while a signal due to 30-COOH of the aglycone remained almost unshifted (δ 179.1 in **4** and δ 179.3 in **7**). This indicated that methylation had occurred at the glucuronide moieties. *Anal.* Calcd for $C_{44}H_{66}O_{16} \cdot 3H_2O$: C, 58.39; H, 8.02. Found: C, 58.30; H, 7.79.

Acetylation of 7——A solution of **7** (244 mg) in Ac_2O–C_5H_5N (1 : 1) (10 ml) was stirred at room temperature for 4 h. The solution was poured into ice water and the precipitate was extracted with $CHCl_3$. The extract was concentrated to dryness and the residue was chromatographed on silica gel [solvent: C_6H_6–acetone (2 : 1)] to give a penta-acetate of **7** (133 mg) as a white powder, $[\alpha]_D^{18}$ +40.8° (c = 1.07, $CHCl_3$). The ^{1}H- and ^{13}C-NMR spectra in C_5D_5N indicated the presence of five acetoxyl groups. *Anal.* Calcd for $C_{54}H_{76}O_{21}$: C, 60.10; H, 7.29. Found: C, 59.71; H, 7.20.

Synthesis of 5——The penta-acetate (5.38 g) of **7**, α-acetobromoglucose (3.31 g) and Ag_2CO_3–Celite (3.51 g) were mixed in anhydrous 1,2-dichloroethane (150 ml). A small amount of the solvent was evaporated off to remove moisture by co-distillation, and then the mixture was heated under reflux for 4 h. The reaction mixture was filtered and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel [solvent: C_6H_6–acetone (6 : 1)] to give a dimethyl ester of the nona-acetate of **5** in a yield of 73%. A white powder, $[\alpha]_D^{24}$ +33.4° (c = 1.02, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.18 (1H, d, J = 8.6 Hz, ester type β-anomeric proton). ^{13}C-NMR ($CDCl_3$) δ: 91.6 (ester type β-anomeric carbon), 100.7, 103.3 (1C each, β-anomeric carbon). *Anal.* Calcd for $C_{68}H_{94}O_{30} \cdot 3H_2O$: C, 56.50; H, 6.97. Found: C, 56.64; H. 6.76.

A solution of the dimethyl ester nona-acetate (5.18 g) in 0.25% methanolic CH_3ONa (400 ml) was stirred at room temperature for 15 min. The mixture was neutralized with Amberlite MB-3 and then concentrated to dryness. The residue was chromatographed on silica gel [solvent: $CHCl_3$–MeOH–H_2O (30 : 10 : 1, homogeneous)] to give a dimethyl ester of **5** in a yield of 56%. A white powder. $[\alpha]_D^{24}$ +63.4° (c = 1.01, MeOH). ^{1}H-NMR (C_5D_5N) δ: 3.81, 3.70 (3H s each, –OCH_3), 6.28 (1H, d, J = 7.1 Hz, ester type β-anomeric proton), 5.29 (1H, d, J = 6.8 Hz, β-anomeric proton), 4.91 (1H, d, J = 6.4 Hz, β-anomeric proton). ^{13}C-NMR (C_5D_5N) δ: 51.9 (2C, –OCH_3 × 2), 95.8 (ester type β-anomeric carbon), 104.8, 106.6 (1C each, β-anomeric carbon). *Anal.* Calcd for $C_{50}H_{76}O_{21} \cdot 5/2H_2O$: C, 56.75; H, 7.72. Found: C, 56.65; H, 7.58.

A solution of this dimethyl ester (201 mg) in 0.2% K_2CO_3 (in 50% EtOH, 30 ml) was stirred at room temperature for 1 h. The mixture was neutralized with Amberlite MB-3 and the product was purified by column chromatography on silica gel [solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)] followed by HPLC to give **5** in a yield of 60%. HPLC conditions: on TSKgel ODS-120T (21.5 mm × 30 cm); mobile phase, 5% AcOH–MeOH–2-PrOH (45 : 50 : 5); flow rate, 6 ml/min; detection, UV 254 and R.I. **5**: A white powder. $[\alpha]_D^{24}$ +72.7° (c = 1.02, MeOH). ^{1}H-NMR (C_5D_5N) δ: 6.27 (1H, d, J = 7.3 Hz, ester type β-anomeric proton), 5.34 (1H, d, J = 6.6 Hz, β-anomeric proton), 4.99 (1H, d, J = 6.6 Hz, β-anomeric proton). ^{13}C-NMR (C_5D_5N) δ: 95.9 (ester type β-anomeric carbon), 105.0, 106.7 (1C each, β-anomeric carbon). *Anal.* Calcd for $C_{48}H_{72}O_{21} \cdot 9/2H_2O$: C, 54.07; H, 7.66. Found: C, 54.10; H, 7.51.

Synthesis of 6——The penta-acetate of **7** (*vide supra*, 9.96 g), the methyl ester of α-acetobromoglucuronic acid[11] (4.0 g) and Ag_2CO_3–Celite (4.15 g) were mixed in anhydrous 1,2-dichloroethane (80 ml). A small amount of the solvent was distilled off to remove moisture by co-distillation and then the mixture was refluxed for 4 h. The mixture was filtered and the product was chromatographed on silica gel [solvent: C_6H_6–acetone (6 : 1)] to give a trimethyl ester of the octa-acetate of **6** in a yield of 43%. Colorless prisms from MeOH–EtOH, mp 264—267 °C. $[\alpha]_D^{18}$ +45.9° (c = 1.04, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.79 (1H, d, J = 7.6 Hz, ester type β-anomeric proton), 4.51 (1H, d, J = 7.3 Hz, β-anomeric proton), 4.18 (1H, d, J = 9.3 Hz, β-anomeric proton). ^{13}C-NMR ($CDCl_3$) δ: 91.2 (ester type β-anomeric carbon), 100.7, 103.3 (1C each, β-anomeric carbon). *Anal.* Calcd for $C_{67}H_{92}O_{30}$: C, 58.42; H, 6.73. Found: C, 58.30; H, 6.78.

A 1% aqueous KOH solution (70 ml) was added to a solution of the trimethyl ester of the octa-acetate (1.01 g) in dioxane (50 ml), and the mixture was stirred at room temperature for 20 min. After deionization by treatment with Amberlite MB-3, the product was purified by chromatography on silica gel [solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1,

homogeneous)] and then on LiChroprep RP-8 (Merck, 40—63 mesh) (solvent: 60% MeOH) followed by HPLC to give **6** in a yield of 12%. Conditions of HPLC; on TSKgel ODS-120T (21.5 mm × 30 cm); mobile phase, 5% AcOH–MeOH–2-PrOH (45 : 50 : 5); flow rate, 6 ml/min; detection, UV 254 nm and R.I. **6**: A white powder. $[\alpha]_D^{24}$ +83.7° (c = 1.03, MeOH). ^{1}H-NMR (C_5D_5N) δ: 6.36 (1H, d, J=7.3 Hz, ester type β-anomeric proton), 5.35 (1H, d, J=6.8 Hz, β-anomeric proton), 5.00 (1H, d, J=5.9 Hz, β-anomeric proton). ^{13}C-NMR (C_5D_5N) δ: 95.9 (ester type β-anomeric carbon), 105.0, 106.8 (1C each, β-anomeric carbon). *Anal.* Calcd for $C_{48}H_{70}O_{22}\cdot 9/2H_2O$: C, 53.37; H, 7.37. Found: C, 53.20; H, 7.14.

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References

1) H. Kimata, N. Sumida, N. Matsufuji, T. Morita, K. Ito, N. Yata and O. Tanaka, *Chem. Pharm. Bull.*, **33**, 2849 (1985).
2) K. Watanabe, H. Fujino, T. Morita, R. Kasai and O. Tanaka, submitted to *Planta Medica*.
3) H. Kimata, T. Nakashima, S. Kokubun, K. Nakayama, Y. Mitoma, T. Kitahara, N. Yata and O. Tanaka, *Chem. Pharm. Bull.*, **31**, 1998 (1983).
4) R. Kasai, H. Fujino, T. Kuzuki, W.-H. Wong, C. Goto, N. Yata, O. Tanaka, F. Yasuhara and S. Yamaguchi, *Phytochemistry*, **25**, 871 (1986).
5) K. Nakayama, H. Fujino, R. Kasai, Y. Mitoma, N. Yata and O. Tanaka, *Chem. Pharm. Bull.*, **34**, 3279 (1986).
6) R. Kasai, M. Miyakoshi, K. Matsumoto, R.-L. Nie, J. Zhou, T. Morita and O. Tanaka, *Chem. Pharm. Bull.*, **34**, 3974 (1986).
7) R. Kasai, M. Miyakoshi, R.-L. Nie, J. Zhou, K. Matsumoto, T. Morita, M. Nishi, K. Miyahara and O. Tanaka, *Phytochemistry*, **27**, 1439 (1988).
8) I. Kitagawa, J.-L. Zhou, T. Taniyama and M. Yoshikawa, Abstracts of Papers, 107th Annual Meeting of the Pharmaceutical Society of Japan, April 1987, p. 337.
9) H. Kimata, R. Kasai and O. Tanaka, *Chem. Pharm. Bull.*, **30**, 4373 (1982).
10) T. Morita, R. Kasai, H. Kohda, O. Tanaka, J. Zhou and T.-R. Yanag, *Chem. Pharm. Bull.*, **31**, 3205 (1983).
11) G. N. Bollenback, J. W. Long, D. G. Benjamin and J. A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).