120°[15]). *Anal.* Calcd. for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56. Found: C, 49.22; H, 5.83; N, 11.58. TPU was synthesized from *p*-toluenesulfonamide and *n*-propyl isocyanate. mp 151° (Lit. 151—152°,[16] 150—151°[17]). *Anal.* Calcd. for $C_{11}H_{16}N_2O_3S$: C, 51.55; H, 6.29; N, 10.93. Found: C, 51.17; H, 6.37; N, 10.77. APU was obtained by hydrolysis of 4-acetylaminobenzenesulfonyl *n*-propylurea which was synthesized from 4-acetylaminobenzenesulfonamide and *n*-propyl isocyanate. mp 135—136°. *Anal.* Calcd. for $C_{10}H_{15}N_3O_3S$: C, 46.68; H, 5.88; N, 16.33. Found: C, 46.53; H, 5.89; N, 16.19. IPU was synthesized from 4-iodobenzenesulfonamide and *n*-propyl isocyanate. mp 155—156° (Lit. 155—156°[18]). *Anal.* Calcd. for $C_{10}H_{13}IN_2O_3S$: C, 32.60; H, 3.50; N, 7.61. Found: C, 32.69; H, 3.48; N, 7.42. CPU was purchased from Toyama Chem. Ind. Tokyo. BSA was purchased from Sigma Chem. Co., St. Louis. The specific activity of ^{35}S labelled CPU which was obtained from The Radiochemical Centre, Amersham, was 13.7 mCi per mM.

Measurements——The concentration of total and free CPU, IPU, APU were determined with a Shimazu double beam spectrophotometer, model UV-200, at 231, 246 and 254 nm, respectively. Those of BPU and TPU were determined with Hitachi double wavelength spectrophotometer model 356 using the wavelength pairs of 220—280 nm and 226—280 nm, respectively.

The dynamic dialysis was carried out in a glass flow cell of which inner volume was 7 ml. The dialyzer flow rate was controled to 0.42 ml per minute. The radio activities of ^{35}S labelled CPU in dialyzers were determined with Aloka liquid scintillation counter model LSC-602 using counting cocktail of PPO (7 g) and POPOP (0.1 g) in a mixture of ethanol (310 ml) and toluene (690 ml).

The pH measurements were carried out with Hitachi-Horiba pH meter model M-7 calibrated with standard buffer solution.

Acknowledgement The authors wish to thank Dr. J.M. Becktel, Midwest Research Support Center, Veterans Administration Hospital, for his kind information on the computer program.

15) F.J. Marshall and M.V. Sigal Jr., *J. Org. Chem.*, **23**, 927 (1958).
16) C. Ruti and A. Stener, *Ann. Chim.* (Rome), **53**, 1370 (1963) [*C.A.*, **60**, 6770*f* (1964)], Z. Brozozowaki, *Rocz. Chem.* (Pol.), **43**, 1761 (1969) [*C.A.*, **72**, 21434*w* (1970)].
17) Y. Nitta, N. Ando, Y. Ikeda, M. Koizumi, and A. Shioya, *Yakugaku Zasshi*, **82**, 191 (1962).
18) G.N. Holcomb, C.M. Boyd, R.E. Counsell, W.H. Beierwaltes, R.A. Szczesnial, D.R.K. Murty, and G.A. Bruno, *J. Pharm. Sci.*, **60**, 390 (1971).

[Chem. Pharm. Bull. 25(12)3408—3409(1977)]

UDC 547.918.02 : 581.192

Pharmacognostical Studies on Gleditsia. III.[1] Flavonoidal Constituents in the Leaves of *Gleditsia japonica* Miquel and *G. sinensis* Lamarck

Masao Yoshizaki,[2a] Tsuyoshi Tomimori,[2b] and Tsuneo Namba[2a]

Research Institute for Wakan-Yaku, University of Toyama[2a] and School of Pharmacy, Hokuriku University[2b]

(Received May 4, 1977)

Six flavonoids; luteolin-7-glucoside, isoquercitrin, vitexin, isovitexin, orientin, and homo-orientin were isolated from the leaves of *Gleditsia japonica* Miquel and *G. sinensis* Lamarck.

Keywords——*Gleditsia japonica* Miquel; *Gleditsia sinensis* Lamarck; flavonoid glycosides; luteolin-7-glucoside; isoquercitrin; vitexin; isovitexin; orientin; homo-orientin

Gleditsia japonica Miquel (Japanese name: Saikachi) is a deciduous tall tree of the family Leguminosae, distributed in Japan, Korea, and China. In preceding paper,[1] it was reported

1) Part II: M. Mitsuno and M. Yoshizaki, *Yakugaku Zasshi*, **77**, 1208 (1957).
2) Location: *a) 3190, Gofuku, Toyama; b) 3, Ho, Kanagawa-machi, Kanazawa.*

that two flavonoids, fisetin and fustin, and a leucoanthocyanidin, mollisacacidin (gleditsin),[3] were isolated from the heartwood of this plant.

The present paper deals with the result of further investigation on the flavonoidal constituents in the leaves of *G. japonica* Miquel and *G. sinensis* Lamarck.

As described in the experimental part, six flavonoids, luteolin-7-glucoside (I), isoquercitrin (II), vitexin (III), isovitexin (IV), orientin (V), and homo-orientin (VI), were isolated from the leaves of both plants. They were identified by direct comparison with authentic specimens.

Experimental

All melting points were uncorrected. Ultraviolet (UV) spectra were determined using diagnostic reagents by standard procedures[4] with a Hitachi Recording Spectrophotometer EPS-2U type. Infrared (IR) spectra were run on KBr disks using a JASCO IR-E Spectrophotometer. Paper chromatography (PPC) was carried out using Toyo Filter Paper No. 50 and solvent systems of 15% AcOH, 60% AcOH, *n*-BuOH–AcOH–H_2O (4:1:5, upper), 85% PhOH, and *n*-BuOH–pyridine–H_2O (10:3:3). Thin-layer chromatography (TLC) was carried out on silica gel G with AcOEt–MeCOEt–HCO_2H–H_2O (5:3:1:1). Spray reagents, for flavonoid: 5% Na_2CO_3 aqueous solution, for sugar: *n*-BuOH solution of aniline hydrogen phthalate.

Extraction and Isolation of Flavonoid——The leaves of *G. japonica* (A) and *G. sinensis* (B) were collected in Sept. at the Botanical Garden for Medicinal Plants, Univ. of Toyama, and in Oct. at School of Medicine, Chiba Univ., respectively. The MeOH extracts from dried leaves of A (700 g) was digested with boiling H_2O and the resulting aqueous solution was extracted with ether. The H_2O layer was passed through a column of Polyamide Woelm (500 g), and chromatographed with MeOH as an eluant monitoring by PPC and TLC. Yield: I (200 mg), II (25 mg), III (200 mg), IV (100 mg), V (50 mg), VI (400 mg).

Dried leaves of B (50 g) were also treated in a similar manner as above, yielded six flavonoids (I—VI).

Luteolin-7-glucoside (I)——Pale yellow powdery crystals, mp 259—260° (dec.), from 30% acetone. *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot 2H_2O$: C, 52.00; H, 4.90. Found: C, 51.75; H, 4.68. It was identical with luteolin-7-glucoside by mixed fusion, PPC, TLC, UV, and IR. Hydrolysis with 5% H_2SO_4 gave luteolin and D-glucose.

Isoquercitrin (II)——Yellow needles, mp 223—224°, from 50% EtOH. *Anal.* Calcd. for $C_{21}H_{20}O_{12}\cdot 3H_2O$: C, 48.65; H, 5.06. Found: C, 48.81; H, 4.87. It was identical with isoquercitrin by mixed fusion, PPC, TLC, UV, and IR. Hydrolysis with 5% H_2SO_4 gave quercetin and D-glucose.

Vitexin (III)——Yellow needles, mp 265° (dec.), from dioxane–H_2O (1:2). *Anal.* Calcd. for $C_{21}H_{20}O_{10}$: C, 58.33; H, 4.66. Found: C, 58.01; H, 4.88. It was identical with vitexin by mixed fusion, PPC, TLC, UV, and IR.

Isovitexin (IV)——Yellow needles, mp 223—224° (dec.), from MeOH–AcOEt. *Anal.* Calcd. for $C_{21}H_{20}O_{10}\cdot H_2O$: C, 56.00; H, 4.92. Found: C, 55.91; H, 4.88. It was identical with isovitexin by mixed fusion, PPC, TLC, UV, and IR.

Orientin (V)——Recrystallization of V from dioxane–H_2O (1:2) yielded yellow needles, which began to sinter at *ca.* 260° and decomposed at 283—285°. *Anal.* Calcd. for $C_{21}H_{20}O_{11}$: C, 56.25; H, 4.50. Found: C, 56.35; H, 4.62. It was identical with orientin by mixed fusion, PPC, TLC, UV, and IR.

Homo-orientin (VI)——Yellow needles, mp 238° (dec.) from 50% EtOH. *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot H_2O$: C, 54.08; H, 4.72. Found: C, 54.39; H, 4.50. It was identical with homo-orientin by mixed fusion, PPC, TLC, UV, and IR.

Interconvertion of III and IV (or V and VI)——Few mg each of III and IV (or V and VI) was respectively boiled with 10% H_2SO_4 (5 ml) for 6 hr. After cooling and dilution with H_2O, the reaction mixture was passed through a column of Polyamide (Woelm) (1 g). The column was washed with H_2O until the eluate was neutral. Subsequent elution of the column with MeOH afforded the mixture of flavonoid. In each case, two spots of flavonoid were revealed on paper and silica gel plate, the *Rf* values of which were found to be the same as those of III and IV (or V and VI) respectively.

Acknowledgement The authors are deeply indebted to Dr. M. Harada, Faculty of Pharmaceutical Sciences, Chiba Univ., for his kindly collecting the leaves of *Gleditsia sinensis*. They also express their gratitude to Mr. H. Hori, Faculty of Pharmaceutical Sciences, Univ. of Toyama, for carrying out the elemental analyses.

3) Gleditsin[1] was found to be identical with mollisacacidin [J.W. Clark-Lewis and M. Mitsuno, *J. Chem. Soc.*, **1958**, 1724].

4) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970, pp. 35—38.