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Isoflavonoids in the Roots of *Thermopsis fabacea* D. C. (Leguminosae)[1]

MUNEHISA ARISAWA, YUKO KYOZUKA, TOSHIMITSU HAYASHI, MINEO SHIMIZU, and NAOKATA MORITA

Pharmaceutical Institute, Toyama Medical and Pharmaceutical University[2]

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Nine isoflavonoids (I—IX) were isolated from the roots of *Thermopsis fabacea* D. C. (Leguminosae). The methanol extract afforded formononetin (I) and daidzein (II), the ethereal extract afforded trifolirhizin (III), *dl*-maackiain (IV), genistein (V), and 7,3′-dihydroxy-4′-methoxyisoflavone (VI), and the ethyl acetate extract afforded formononetin-7-O-*β*-D-glucoside (VII), daidzin (VIII), and genistin (IX). These compounds were identified by means of degradation, spectroscopic studies, and direct comparison with authentic samples.

Keywords——*Thermopsis fabacea* D. C.; trifolirhizin; formononetin-7-O-*β*-D-glucoside; daidzin; genistin; 7,3′-dihydroxy-4′-methoxyisoflavone

The constituents of *Thermopsis fabacea* D. C. (*Leguminosae*) were examined, and we report here the isolation and identification of nine isoflavonoids (I—IX) from the roots.

The crude precipitate from the methanol extract was chromatographed on a column to give I, mp 264—265°, and II, mp over 300°. The ultraviolet (UV) maxima of both I (240 (s), 248, 259 (s), 301 nm) and II (238 (s), 249, 259 (s), 303 (s) nm) were unchanged upon addition of aluminum chloride. I gave a monoacetate (Ia), mp 173—174°, and II gave a diacetate (IIa), mp 182—184°, under usual acetylation conditions. In the mass spectra of Ia and IIa, the molecular ion peaks were observed at *m*/*e* 310 and *m*/*e* 338, respectively. I and II appeared to be isoflavones and were identified as formononetin and daizein, respectively, by direct comparison with authentic samples.

The crude solid from the ethereal extract was purified by recrystallization to give colorless needles (III), mp 141°, $[\alpha]_D$ −185°. The UV absorption spectrum of III showed maxima at 280, 284, 309 nm and was similar to that of pterocarpin derivatives. III gave a tetraacetate (IIIa), mp 187° under usual acetylation conditions. In the mass spectrum of IIIa, the molecular ion peak was confirmed at *m*/*e* 614 and fragment peaks were observed at *m*/*e* 331 and 284. In the proton nuclear magnetic resonance (PMR) spectrum of a trimethylsilyl (TMS) ether of III, a 2H singlet at 5.93 ppm indicated a methylenedioxy group, and a 1H doublet (J=6.5 Hz) centered at 4.85 ppm was attributed to the anomeric proton of a *β*-linked sugar. Hydrolysis of III with hydrochloric acid afforded glucose and an aglycone, mp 191—195°, $[\alpha]_D$ ±0, which was identical with authentic *d*-maackiain on infrared (IR) spectral comparison. III was identified as trifolirhizin[3] by direct comparison with an authentic specimen.

The filtrate obtained above afforded IV, V, and VI, respectively. IV, mp 191—195°, was identical with the aglycone of III (specific optical rotation and UV and IR spectral comparison). V, mp 285—290°, was thought to be genistein based on its physical properties, and was identified by direct comparison with an authentic sample. VI, mp 251—253°, showed a molecular ion peak at *m*/*e* 284 in the mass spectrum, and afforded a diacetate (VIa), mp 205—208°.

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1) Presented in part at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980. This paper is part IX of Studies on Unutilized Resources. Part VIII: M. Arisawa, M. Fukuta, M. Shimizu, and N. Morita, *Chem. Pharm. Bull.*, **27**, 1252 (1979).

2) Location: *Sugitani 2630, Toyama 930-01, Japan.*

3) S. Shibata and Y. Nishikawa, *Chem. Pharm. Bull.*, **11**, 167 (1963).

It was anticipated that VI was 7,3'-dihydroxy-4'-methoxyisoflavone[4] based on its physical properties, and this was confirmed by direct comparison with an authentic specimen.

The ethyl acetate extract was chromatographed on a column to give VII, VIII, and IX, respectively. VII, colorless prisms, mp 223°, showed UV maxima at 250 (s), 259, and 301 (s) nm, and gave a crystalline acetate, mp 189—191° (M^+ 598). Hydrolysis of VII gave D-glucose and an aglycone which was identical with I. In the PMR spectrum of VII-TMS, a 1H doublet ($J=6.0$ Hz) centered at 4.91 ppm was attributed to the anomeric proton of a β-glucosyl moiety. Therefore, VII was identified as formononetin-7-O-β-D-glucoside. VIII, mp 235°, and IX, mp 249°, were hydrolyzed with hydrochloric acid to afford D-glucose and aglycones that were identical with II and V, respectively. VIII and IX were identified as daidzin and genistin, respectively, by direct comparison with authentic samples.

Experimental

All melting points are uncorrected and were taken on a Yanaco micro mp apparatus, model MP-3. IR spectra were recorded as KBr tablets on a JASCO IR-S spectrometer and UV spectra were measured with a Hitachi spectrophotometer, model 124. PMR spectra were obtained on Hitachi Perkin-Elmer R-24B and Varian EM-390 NMR spectrometers, and the signals are given as chemical shifts in δ values (ppm) with TMS as an internal standard. The following abbreviations are used: s=singlet, d=doublet, dd=double doublet, m=multiplet, br=broad. Mass spectra were obtained with a JEOL JMS-OISG-2 mass spectrometer. Specific optical rotation was determined with a JASCO-DIP-4 spectrometer.

Extraction and Separation——Fresh roots of the materials were cut and extracted with hot MeOH. Removal of MeOH by evaporation afforded the precipitate. The precipitate was chromatographed on a silica gel column to afford two kinds of colorless needles, mp 264—265° (I) and mp over 300° (II), upon elution with a PB-AcOEt mixture. The filtrate was extracted with Et_2O and then AcOEt. The ethereal extract was crystallized from MeOH to afford a crude solid. The crude solid was recrystallized from MeOH to afford colorless silky needles. mp 141° (III). The filtrate of the crude solid afforded I, II, III, and colorless prisms, mp 191—195° (IV) upon silica gel column chromatography with a mixture of $CHCl_3$–MeOH, and also gave two compounds, mp 285—290° (V) and mp 251—253° (VI), upon preparative TLC. The AcOEt extract was chromatographed on a silica gel column with $CHCl_3$–MeOH mixture to afford three substances, mp 223° (VII), mp 235° (VIII), and mp 249° (IX).

Formononetin (I)——Colorless needles, mp 264—265°. UV λ_{max}^{MeOH} nm: 240 (s), 248, 259 (s). UV $\lambda_{max}^{MeOH-AlCl_3}$ nm: unchanged. MS (*m/e*): 268 (M^+), 137, 132. Mixed mp tests and IR spectral comparison revealed this compound to be identical with authentic formononetin.

I Acetate (Ia): Acetic anhydride was added to a solution of I in pyridine. After being heated on a water bath for 3 hr, the reaction mixture was worked up in the usual manner. The crude acetate was chromatographed on a silica gel column and recrystallized from MeOH to give colorless needles, mp 173—174. MS (*m/e*): 310 (M^+), 268, 137, 132.

Daidzein (II)——Colorless needles, mp over 300°. UV λ_{max}^{MeOH} nm: 238 (s), 249, 259 (s), 303 (s). UV $\lambda_{max}^{MeOH-AlCl_3}$ nm: unchanged. MS (*m/e*): 254 (M^+), 137, 118. IR spectral comparison showed this compound to be identical with authentic daidzein.

II Acetate (IIa): II was acetylated with acetic anhydride and pyridine in the usual manner to afford colorless needles, mp 182—184°. MS (*m/e*): 338 (M^+), 296, 254, 137, 118.

Trifolirhizin (III)——Colorless silky needles, mp 141°, $[\alpha]_D$ −185° (MeOH), UV λ_{max}^{MeOH} nm: 280, 284, 309. PMR (TMS ether of III, in CCl_4): 3.2—3.7 (8H, br, aliphatic H), 4.20 (1H, dd, $J=3.0$ Hz, $J=9.0$ Hz, C_{6a}–H), 4.85 (1H, d, $J=6.5$ Hz, glucosyl anomeric H), 5.42 (1H, d, $J=6.0$ Hz, C_{11a}–H), 5.93 (2H, s, methylenedioxy H), 6.40 (1H, s, C_7–H), 6.55 (1H, d, $J=2.0$ Hz, C_4–H), 6.63 (1H, dd, $J=2.0$ Hz, $J=8.5$ Hz, C_2–H), 6.65 (1H, s, C_{10}–H), 7.39 (1H, d, $J=8.5$ Hz, C_1–H). Mixed mp tests and IR spectral comparison showed this compound to be identical with authentic trifolirhizin.

III Acetate (IIIa): III was acetylated with acetic anhydride and pyridine in the usual manner to give colorless needles, mp 187°. MS (*m/e*): 614 (M^+), 331, 284, 271, 229, 211, 169, 109. III was hydrolyzed with c. HCl over an open flame. The hydrolyzed mixture was diluted with H_2O and extracted with Et_2O. The ethereal extract was chromatographed on a silica gel column to give IV. The H_2O layer was evaporated to dryness for examination of sugars. PPC *Rf*: 0.19 (brown) (*n*-BuOH–AcOH–H_2O (4:1:2), glucose 0.19 (brown)). TLC (plate: silica gel containing 0.1 N boric acid) *Rf*: 0.66 (brown) (C_6H_6–AcOH–MeOH (1:1:3), glucose 0.66 (brown)). Color reaction with 0.1 N aniline hydrogen phthalate.

***dl*-Maackiain (IV)**——Colorless prisms, mp 191—195°. Acetate mp 158—160°. $[\alpha]_D$ ±0. UV λ_{max}^{MeOH} nm:

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[4] M.R. Parthasarathy, R.N. Puri, and T.R. Seshadri, *Indian J. Chem.*, 7, 1817 (1967).

280, 286, 309. The IR spectrum of IV was superimposable on that of an authentic *d*-maackiain.

Genistein (V)——Colorless needles, mp 285—290°, with a purplish-brown color reaction to $FeCl_3$. UV λ_{max}^{MeOH} nm: 261, 328 (s). MS (*m/e*): 270 (M^+), 242, 153, 152, 118. Mixed mp tests and IR spectral comparison revealed this substance to be identical with authentic genistein.

V Acetate (Va): Colorless needles, mp 208—210° MS (*m/e*): 396 (M^+), 395, 354, 312, 270, 153, 152, 118.

7,3′-Dihydroxy-4′-methoxyisoflavone (VI)——Colorless needles, mp 251—253°. UV λ_{max}^{MeOH} nm: 247, 260 (s). 290, 308 (s). MS (*m/e*): 284 (M^+), 148, 137. Mixed mp tests and IR spectral comparison showed this compound to be identical with authentic 7,3′-dihydroxy-4′-methoxyisoflavone.

VI Acetate (VIa): Colorless needles, mp 205—208° MS (*m/e*): 368 (M^+), 326, 284, 148, 137

Formononetin-7-O-β-glucoside (VII)——Colorless prisms, mp 223° UV λ_{max}^{MeOH} nm: 250 (s), 259, 301 (s), PMR (TMS ether of VII, in CCl_4): 3.18—3.83 (9H, m, aliphatic H+OMe), 4.91 (1H, d, $J=6.0$ Hz, glucosyl anomeric H), 6.88 (2H, d, $J=9.0$ Hz, $C_{3',5'}$–H), 6.93 (1H, d, $J=2.0$ Hz, C_8–H), 6.98 (1H, dd, $J=2.0$ Hz, $J=9.0$ Hz, C_6–H), 7.46 (2H, d, $J=9.0$ Hz, $C_{2',6'}$–H), 7.85 (1H, s, C_2–H), 8.16 (1H, d, $J=9.0$ Hz, C_5–H). VII was hydrolyzed with c. HCl afforded I, and glucose was detected from the filtrate of I by PPC and TLC.

VII Acetate: Colorless needles, mp 189—191°. MS (*m/e*): 598 (M^+), 331, 271, 267, 229, 211, 169, 132, 109.

Daidzin (VIII)——Colorless needles, mp 235°. UV λ_{max}^{MeOH} nm: 256, 310 (s). VIII was hydrolyzed with c. HCl to give II and glucose. Mixed mp tests and IR spectral comparison showed this substance to be identical with authentic daidzin.

Genistin (IX)——Colorless needles, mp 249°, with a purplish-brown color reaction to $FeCl_3$. UV λ_{max}^{MeOH} nm: 228 (s), 260, 305 (s). IX was hydrolyzed with c. HCl to afford V and glucose. Mixed mp tests and IR spectral comparison revealed this compound to be identical with authentic genistin.

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Synthesis of 3-(Guaiazulen-3-yl)-3-oxopropionic Acid Derivatives[1]

YOSHIAKI MUTO, HIROMI ICHIKAWA, and KANAME TAKAGI

Research Laboratories, Zeria Pharmaceutical Co., Ltd.[2]

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Guaiazulene (1,4-dimethyl-7-isopropylazulene) reacted with malonyl dichloride in the absence of a Lewis acid to afford 3-(guaiazulen-3-yl)-3-oxopropionylchloride (**1**), which was converted by treatment with water into 3-(guaiazulen-3-yl)-3-oxopropionic acid (**2**) and 3-acetylguaiazulene (**3**). Upon treatment of **1** with some alcohols and aromatic amines, the corresponding esters (**4**) and amides (**5**) were obtained, respectively. The reaction of methyl 3-(guaiazulen-3-yl)-3-oxopropionate (**4a**) with hydrazine gave 3-(guaiazulen-3-yl)-3-oxopropionylhydrazide (**6**), which was easily cyclized into 5-(guaiazulen-3-yl)-2,3-dihydropyrazol-3-one (**7**).

Keywords——guaiazulene; malonyl dichloride; acylation of guaiazulene; 3-(guaiazulen-3-yl)-3-oxopropionic acid derivatives; 5-(guaiazulen-3-yl)-2,3-dihydropyrazol-3-one

It is known that the acylation of guaiazulene (1,4-dimethyl-7-isopropylazulene) with acid halides should be carried out in the presence or absence of Lewis acid, depending on the acid

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[2] Location: *2-9-10, Funado, Itabashi-ku, 174, Tokyo.*