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The Constituents of *Cirsium japonicum* D.C. var. *takaoense* KITAMURA[1] Isolation of Two New Flavonoids, Cirsitakaoside (IV) and Cirsitakaogenin (VI)

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Two new flavone, cirsitakaoside (IV), $C_{23}H_{24}O_{11}$, mp 246—247°, and cirsitakaogenin (VI), $C_{17}H_{14}O_6$, mp 259—260°, have been isolated from the fresh leaves of *Cirsium japonicum* D.C. var. *takaoense* KITAMURA (Compositae) together with pectolinarin (I). The structures of cirsitakaoside (IV) and cirsitakaogenin (VI) have been determined as 5,7-dihydroxy-8,4′-dimethoxyflavone-7-O-β-D-glucoside and 5,7-dihydroxy-8,4′-dimethoxyflavone, by chemical degradation and spectral means, respectively.

Keywords——Compositae; *Cirsium japonicum* D.C. var. *takaoense* KITAMURA; pectolinarin; cirsitakaoside; 5,7-dihydroxy-8,4′-dimethoxyflavone-7-O-β-D-glucoside; cirsitakaogenin; 5.7-dihydroxy-8,4′-dimethoxyflavone

From the standpoint of chemotaxonomical studies, we have previously reported on the constituents of the Formosan *Cirsium*.[1,3–5]

As the continuation of this series, we now wish to report pectolinarin (I) and two new constituents, which have been named cirsitakaoside (IV) and cirsitakaogenin (VI), isolated from the methanolic extract of the fresh leaves of *C. japonicum* D.C. var. *takaoense* KITAMURA (Compositae).

The methanolic extract was chromatographed on a polyamide column. Pectolinarin (I), identified by direct comparison with authentic sample, cirsitakaoside (IV), and cirsitakaogenin (VI), were eluted from the column with water, water–methanol (3:1), and methanol, respectively.

Cirsitakaoside (IV), mp 246—247°, was recrystallized from methanol as pale yellow needles and analyzed for $C_{23}H_{24}O_{11}$ by elemental analysis. Compound IV exhibited yellow to Mg-HCl and Zn-HCl, greenish brown to ferric chloride solution, positive reaction to Fehling's solution, and negative color test to Gibbs reagent and gossypetone reaction.[6] The

I : R=rutinose
III: R=H

IV: R′=glucose
VI: R′=H

Chart 1

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[1] This paper is Studies on The Constitutents of Formosan *Cirsium* Species (Part V). Part IV: N. Morita and C.N. Lin, *J. Taiwan Pharm. Assoc.*, **28** 40 (1976).

[2] Location: *a) No. 19, Shin-Chuan 1st Road, Kaohsiung, Taiwan*; *b) Gofuku 3190, Toyama, 930, Japan.*

[3] C.N. Lin and T.S. Wu, *Chemistry* (*The Chinese Chem. Soc., Taiwan, China*), **1972**, 131.

[4] C.N. Lin, C.H. Chang and T.S. Wu, *J. Chinese Chem. Soc.*, **22**, 53 (1975).

[5] C.N. Lin, *J. Chinese Chem. Soc.*, **22**, 275 (1975).

[6] A.G. Perkin, *J. Chem. Soc.*, **103**, 650 (1913).

Table I. Chemical Shifts of C_3, C_6 and C_8–H on Flavones (δ (ppm) in $CDCl_3$)

	C_3–H	C_6–H	C_8–H
Pectolinarin (I)[a]	6.30		6.65
Cirsitakaoside (IV)[a]	6.27	6.48	
Pectolinarin acetate (II)	6.46		7.03
Cirsitakaoside acetate	6.47	6.85	
Pectolinarigenin acetate	6.49		7.20
Cirsitakaogenin acetate (VII)	6.53	6.87	
Baicalein acetate	6.54		7.39
Woogonin acetate	6.62	6.77	

a) TMS ether (in CCl_4).

ultraviolet (UV) absorption spectrum showed maxima at 250 (sh), 276, 323 nm (log ε 4.03, 4.34, 4.37). The UV maxima changed to 260, 290 (sh), 302, 349 nm by addition of aluminum chloride, while unchanged on addition of sodium acetate. The infrared (IR) spectrum of IV exhibited absorption bands at 3250 (hydroxyl), 1660 (carbonyl), and 1610 cm^{-1} (aromatic). The above data clearly indicated that IV belongs to a 5-hydroxyflavone glycoside.

The proton nuclear magnetic resonance (PMR) spectrum of trimethylsilyl (TMS) ether of IV taken in carbon tetrachloride showed a pair of 2H doublets (J=9.0 Hz), centered at 7.70 and 6.97 ppm assignable to the proton located at the 2′, 6′ and 3′, 5′-position, and two 1H singlets at 6.48 and 6.27 ppm assignable to the proton located at 6 and 3-position, respectively (Table I). The 1H doublet (J=6.0 Hz), centered at 4.88 ppm, attributed to the glucose anomeric proton and these data indicated IV may belong to C-6 or C-8 substituted flavonoid 7-O-β-glucoside.[7] Two 3H singlets at 3.90 and 3.70 ppm attributed to the proton of two methoxyl groups. The signals of 5H at 3.07—3.67 ppm attributed to the aliphatic proton.

Acetylation of IV afforded colorless needles, mp 140—141° (IV acetate), and had the composition, $C_{33}H_{34}O_{16}$ by elemental and mass spectral analyses. In the PMR spectrum of IV acetate, two 3H singlets at 3.97 and 3.85 ppm indicated two methoxyl groups, and the 3H and 12H singlets at 2.47 and 2.07 ppm attributed to one aromatic acetyl and four aliphatic acetyl groups, respectively.

Hydrolysis of IV with hydrochloric acid afforded glucose and an aglycone which was identical with cirsitakaogenin (VI) as described below.

Cirsitakaogenin (VI), was recrystallized from methanol as pale yellow needles with mp 259—260° (dec.), and showed the empirical formula $C_{17}H_{14}O_6$, which was confirmed by elemental analysis and mass spectral measurement (M^+ 314). The UV absorption spectrum showed maxima at 274, 332 nm (log ε 4.12, 4.32) and it changed to 264 (sh), 292 (sh), 301, 360 nm by addition of aluminum chloride, and to 272, 335, 385 nm by addition of sodium acetate. From above data, VI has a 5,7-dihydroxyflavone nucleus. VI gave an acetate (VII), $C_{21}H_{18}O_8$, mp 186—187°, under usual acetylation. The PMR spectrum of VII showed a pair of 2H doublets (9.0 Hz), centered at 7.86 and 7.21 ppm assignable to the proton located at 2′,6′ and 3′,5′-position, and two 1H singlets 6.87 and 6.53 ppm assignable to the proton at 6 and 3-position, respectively (Table I). Four 3H singlets at 3.99, 3.87, 2.49, and 2.33 ppm were attributed to two methoxyl groups, and two acetyl groups, respectively. From these findings, the structure of cirsitakaogenin (VI) is best represented as 5,7-dihydroxy-8,4′-dimethoxyflavone.

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[7] T.J. Marbry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970, p. 269.

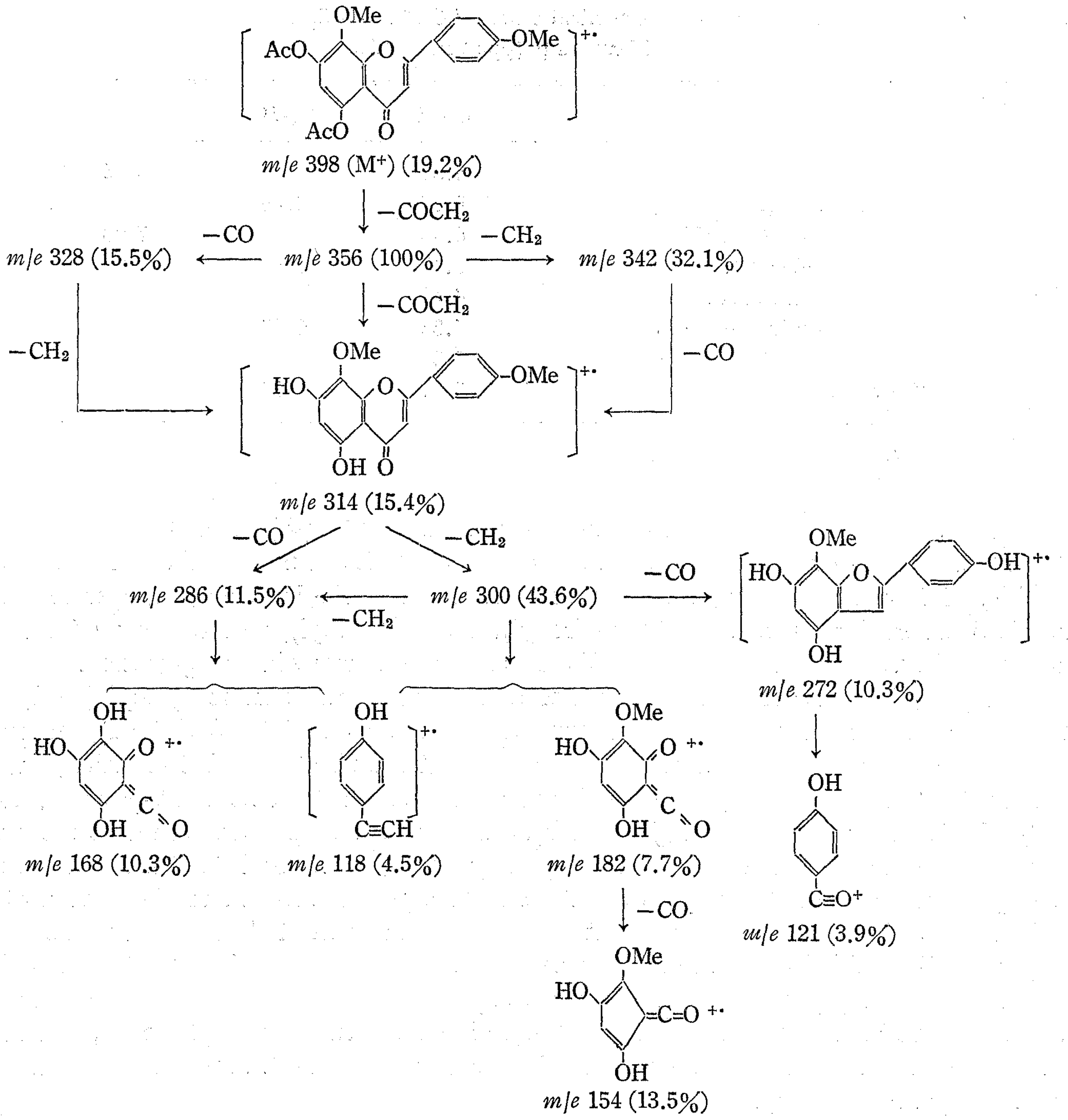

Chart 2. Fragmentation in Mass Spectrum of Cirsitakaogenin Acetate (VII)

The fragmentation pattern of diacetate (VII) in the mass spectrum (Chart 2) clearly substantiated the proposed structure of VI.[8]

Therefore the cirsitakaoside (IV) is 5,7-dihydroxy-8,4′-dimethoxyflavoneglucoside. The bathochromic shifts in UV spectrum of both IV and VI on addition of aluminum chloride and a positive shift of VI with sodium acetate in band I clearly indicated the glycosyl linkage should be bonded at 7-position.

In the PMR spectrum of trimethylsilyl cirsitakaoside, the anomeric proton signal showed a doublet (J=6.0 Hz) at 4.88 ppm which can be attributed to the β-configuration. Consequently, the structure of cirsitakaoside (IV) was determined as 5,7-dihydroxy-8,4′-dimethoxy-7-O-β-D-glucoside. For further, the confirmation of the proposed structure of cirsitakaogenin (VI) by synthesis is now under investigation.

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8) Q.N. Porter and J.Baldas, "Mass Spectrometry of Heterocyclic Compounds," Interscience, Inc. New York-London-Sydney-Toronto, 1971, p. 172.

Experimental[9]

Extraction and Separation——The fresh leaves of *Cirsium japonicum* D.C. var. *takaoense* KITAMURA (2.58 kg), were collected at Ali, Wu-Tai Shian, Ping-Tung Hsien, Taiwan, on July, 1977, chipped and extracted with hot methanol. Evaporation of methanol yielded aqueous extract. The extract was chromatographed on polyamide column. The column was eluted with water, water–methanol (3: 1), and methanol to afford pectolinarin (I), cirsitakaoside (IV), and cirsitakaogenin (VI), respectively.

Pectolinarin (I)——Recrystallization from MeOH gave colorless needles, mp 254—255° (dec.), greenish brown to $FeCl_3$, reddish orange to Mg-HCl, reddish purple to Zn-HCl, and brown under UV light. PPC *Rf*: 0.50 (15% AcOH), 0.79 (30% AcOH). UV λ_{max}^{EtOH} nm: 275, 330. PMR (TMS ether of I, 10% solution in CCl_4) δ (ppm): 0.99 (3H, broad (b.), rhamnosyl CH_3), 3.1—4.0 (16H, b., (3.73 (3H, singlet (s.), OMe), 3.82 (3H, s., OMe)), 4.33 (1H, doublet (d.), $J=2.0$ Hz, rhamnosyl anomeric H), 5.13 (1H, multiplet (m.). glucosyl anomeric H), 6.30 (1H, s., C_3-H), 6.65 (1H, s., C_8-H), 6.88 (2H, d., $J=9.0$ Hz $C_{3',5'}$-H), 7.70 (2H, d., $J=9.0$ Hz, $C_{2'6'}$-H). It was proved as pectolinarin (I) by mixed mp and comparison of IR spectra (KBr) with authentic sample.

Pectolinarin Acetate (II)——PMR (10% solution in $CDCl_3$) δ (ppm): 1.17 (3H, d., $J=6.0$ Hz, rhamnosyl CH_3), 1.85 (3H, s,. $C_{2''}$-OAc), 2.00, 2.02, 2.05 (total 15H, aliphatic OAc × 5) 2.45 (3H, s., aromatic OAc), 3.80 (3H, s., OMe), 3.87 (3H, s., OMe); 6.46 (1H, s., C_3-H), 6.93 (2H, d., $J=9.0$ Hz, $C_{3'5'}$-H), 7.03 (1H, s., C_8-H), 7.74 (2H, d., $J=9.0$ Hz, $C_{2'6'}$-H).

Cirsitakaoside (IV)——Pale yellow needles (MeOH), mp 241—247° (dec.), yellow to Mg-HCl and Zn-HCl, greenish brown to $FeCl_3$, positive reaction to Fehling's solution, negative color test to Gibbs reaction and Gossypeton reaction, and brown under UV light. PPC *Rf*: 0.42 (15% AcOH, 0.64 (30% AcOH). *Anal* Calcd. for $C_{23}H_{24}O_{11}$: C, 57.98; H, 5.08. Found: C, 57.83; H, 5.14. UV λ_{max}^{MeOH} nm (log ε): 250 (sh) (4.03), 276 (4.34), 323 (4.37). UV $\lambda_{max}^{MeOH-AlCl_3}$ nm: 260, 290 (sh), 302, 349. IR ν_{max}^{KBr} cm^{-1}: 3250, 1660, 1610, *etc.* PMR (TMS ether of IV, 10% solution in CCl_4) δ (ppm): 3.07—3.67 (5H, b., aliphatic H), 3.70 (3H, s., OMe), 3.90 (3H, s., OMe), 4.88 (1H, d., $J=6.0$ Hz glucosyl anomeric H), 6.27 (1H, s., C_3-H), 6.48 (1H, s., C_6-H), 6.97 (2H, d., $J=9.0$ Hz, $C_{3'5'}$-H), 7.70 (2H, d., $J=9.0$ Hz, $C_{2'6'}$-H).

Cirsitakaoside Acetate (V)——To a solution of IV in pyridine was added acetic anhydride. After heating on water bath for 3 hr, the reaction mixture worked up as usual manner. Recrystallization from MeOH gave colorless needles, mp 140—141°, and no color to $FeCl_3$. *Anal.* Calcd. for $C_{33}H_{34}O_{16}$: C, 57.70; H, 4.99. Found: C, 57.78; H, 4.90. MS *m/e*: 686 (M^+). PMR (10% solution in $CDCl_3$) δ (ppm): 2.07 (12H, s., aliphatic OAc × 4), 2.47 (3H, s., aromatic OAc), 3.85 (3H, s., OMe), 3.97 (3H, s., OMe), 6.47 (1H, s., C_3-H), 6.85 (1H, s., C_6-H), 7.05 (2H, d., $J=9.0$ Hz, $C_{3',5'}$-H), 7.76 (2H, d., $J=9.0$ Hz, $C_{2',6'}$-H).

Hydrolysis of Cirsitakaoside (IV)——A solution of IV in conc. HCl was heated over an open flame for 3 min. The reaction mixture washed with water and the water insoluble part recrystallized from MeOH to give yellow needles, mp 259—260° (dec.), negative to Fehling's solution. The mixed mp and IR (KBr) spectral comparison with authentic cirsitakaogenin (VI) was identical.

The water soluble part was evaporated to dryness for examination of sugar. PPC *Rf*: 0.18 (brown) (4: 1: 2, glucose 0.18 (brown)). TLC (Plate: Silica gel contained 0.1 N boric acid) *Rf*: 0.67 (brown) (benzene–acetic acid–MeOH (1: 1: 3), glucose 0.67 (brown)). Color reaction with 0.1 N aniline hydrogen phthalate.

Cirsitakaogenin (VI)——Pale yellow needles (MeOH), mp 259—260° (dec.), yellow to Mg-HCl, orange-yellow to Zn-HCl, greenish brown to $FeCl_3$, negative to Gibbs reagent, gossypeton reaction and Fehling's solution, and brown under UV light. PPC *Rf*: 0.14 (15% AcOH), 0.48 (30% AcOH). *Anal.* Calcd. for $C_{17}H_{14}O_6$: C, 64.96; H, 4.49. Found: C, 65.03; H, 4.45. MS *m/e*: 314 (M^+). UV λ_{max}^{MeOH} nm (log ε): 274 (4.12), 332 (4.32). UV $\lambda_{max}^{MeOH-AlCl_3}$ nm: 264 (sh), 292 (sh), 301, 360, UV $\lambda_{max}^{MeOH-NaOAc}$ nm: 272, 335, 385. IR ν_{max}^{KBr} cm^{-1}: 3090, 1655, 1605, *etc.*

Cirsitakaogenin Acetate (VII)——Acetylation of VI with Ac_2O and pyridine in the usual manner gave diacetate, mp 186—187°. *Anal.* Calcd. for $C_{21}H_{18}O_8$: C, 63.30; H, 4.56. Found: C, 63.40; H, 4.52. MS *m/e*: 398 (M^+), 356, 342, 328, 314, 300, 286, 272, 182, 168, 154, 121, 118. PMR (10% solution in $CDCl_3$) δ (ppm): 2.33 (3H, s., aromatic OAc), 2.49 (3H, s., aromatic OAc), 3.87 (3H, s., OMe), 3.99 (3H, s., OMe), 6.53 (1H, s., C_3-H), 6.87 (1H, s., C_6-H), 7.21 (2H, d., $J=9.0$ Hz, $C_{3'5'}$-H), 7.86 (2H, d., $J=9.0$ Hz, $C_{2',6'}$-H).

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[9] All melting points are uncorrected and were taken on a Yanagimoto micro melting apparatus MP-3. IR and UV spectra were recorded on a Japan Spectroscopic Co., Spectrophotometer, Model IR-S and on a Hitachi Spectrophotometer, Model 124, respectively. Mass and PMR spectra were obtained on a Japan Electron Optics Lab., JMS-OISG-2 and JNM-PMX60, respectively. Chemical shifts were recorded as δ values (ppm) with tetramethylsilane (TMS) internal standard.