Chem. Pharm. Bull. 26(10)3071—3074(1978)

UDC 547.972.3.02 : 581.192

The Constituents of *Osmunda* spp. II.[1] A New Flavonol Glycoside of *Osmunda asiatica*

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(Received April 21, 1978)

A novel flavonol glycoside, mp 182—184°, named asiaticalin (I) was isolated from the fresh trophophyll of *Osmunda asiatica* Ohwi (Osmundaceae). The structure of I was established as being kaempferol 3-β-alloside. This is the first found alloside of flavonoid, ^{13}C-nuclear magnetic resonance spectrum of I was discussed in comparison with those of kaempferol (II), and astragalin (X) isolated from the same plant.

Keywords——*Osmunda asiatica*; Osmundaceae; fresh trophophyll; flavonol glycoside; asiaticalin; kaempferol 3-alloside; ^{13}C-NMR

As a part of chemotaxonomical investigations on ferns, the chemical constituents of fresh trophophyll of *Osmunda asiatica* Ohwi and *Osmunda japonica* Thunberg (Osmundaceae) grown in Japan were examined to isolate several fatty acids, sterols, and flavonoids from the crude methanol extracts.[3]

This report deals with the structure of a flavonol glycoside, asiaticalin, isolated from *Osmunda asiatica* Ohwi. Asiaticalin (I), $C_{21}H_{20}O_{11}$, mp 182—184°, gave positive reactions of ferric chloride and magnesium-hydrochloric acid.

On acid hydrolysis, asiaticalin (I) afforded kaempferol (II) as an aglycone, mp 275—277°.

In the ^{1}H-nuclear magnetic resonance (NMR) spectrum of asiaticalin (I), complex signals appeared at δ 3.2—3.9 correspond to six protons attached to C-2″, C-3″, C-4″, C-5″, and C-6″ of the sugar moiety, and the signals at δ 3.9—5.1 which disappeared on addition of deuterium oxide were attributed to four hydroxyls attached to the sugar. A doublet signal at δ 5.68 (J=8.0 Hz) which was not exchanged with deuterium oxide was assigned to an anomeric proton. In the aromatic proton region, a pair of doublet signals at δ 6.12 and 6.42 (J=2.0 Hz) are attributed to $C_{(6)}$-H and $C_{(8)}$-H on the ring A, and a pair of doublets (A_2B_2 type), δ 6.86 and 8.08 (J=9.0 Hz) are attributed to four protons at $C_{(3')}$ and $C_{(5')}$, $C_{(2')}$ and $C_{(6')}$ on the ring B which possesses a hydroxyl group at the C-4′ position.

The presence of a free hydroxyl group at $C_{(7)}$ in asiaticalin (I) was proved by the characteristic bathochromic shift of ultraviolet (UV) absorption by the addition of sodium acetate, and a free hydroxyl group at $C_{(5)}$ was also shown by the same effect on addition of aluminum chloride.

This finding was supported by the experiments of methylation of asiaticalin (I) with diazomethane. The reaction mixture was separated by chromatography to give three compounds, IV, mp 233—235°, V, mp 230—231°, and VI, mp 212—214.5°. The structures of these compounds were substantiated with infrared (IR) and ^{1}H–NMR spectra, and UV spectrum which revealed the similar behaviours as mentioned above. On acid hydrolysis,

1) Part II in the series of "Studies on the Chemotaxonomy of Pteridophyta" Part I: K. Koyama, F. Fuke (Née Sato), J. Kimura, and T. Okuyama, *Shoyakugaku Zasshi*, 32, 126 (1978).
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3) K. Koyama, F. Fuke (Née Sato), J. Kimura, and T. Okuyama, *Shoyakugaku Zasshi*, 32, 126 (1978).

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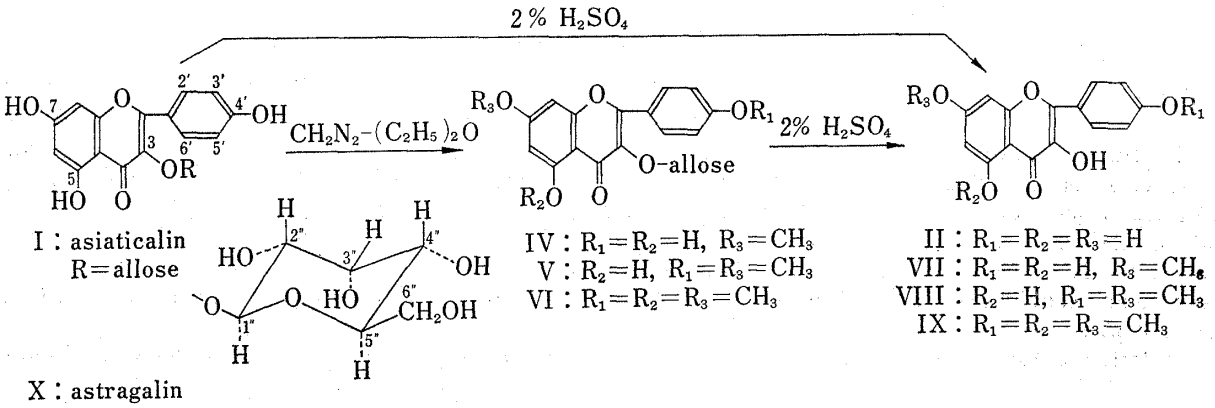
these compounds yielded corresponding methyl ethers of the aglycone (VII, VIII, and IX), which were identified respectively with 7-O-methyl kaempferol, 4′,7-O-dimethyl kaempferol, and 4′,5,7-O-trimethyl kaempferol by a mixed fusion with authentic samples.

The correlations of the chemical shift and the coupling constant of the (C-1″) proton in the ^{1}H–NMR of pyranose to the configuration (α or β) of the anomeric center have been established.[4] Asiaticalin (I) showed a chemical shift at δ 5.68 with a large coupling constant (J=8.0 Hz) to indicate that the hydrogen atoms at $C_{(1'')}$ and $C_{(2'')}$ are in a *trans* diaxial conformation, namely the configulation of the anomeric center was established to be β.

On the basis of the above evidence, asiaticalin (I) was shown to be a flavonol glycoside having an aldohexose (glucose, galactose, gulose, or allose) at $C_{(3)}$ hydroxyl group of kaempferol (II) with a β linkage.

The possibility of presence of glucose or galactose as the sugar moiety of asiaticalin (I) was excluded by the direct comparison of asiaticalin (I) with astragalin (=kaempferol 3-glucoside) (X) and trifolin (=kaempferol 3-galactoside) (XI). Finally, the sugar component (III) of asiaticalin (I) was determined to be allose by paper chromatography, thin layer chromatography, and sugar analyzer using β-D-allose[5] and gulose[6] as the reference samples.

It is interesting to note that allose was found for the first time as the sugar component of flavonoid.



I : asiaticalin R=allose

IV : R_1=R_2=H, R_3=CH_3
V : R_2=H, R_1=R_3=CH_3
VI : R_1=R_2=R_3=CH_3

II : R_1=R_2=R_3=H
VII : R_1=R_2=H, R_3=CH_3
VIII : R_2=H, R_1=R_3=CH_3
IX : R_1=R_2=R_3=CH_3

X : astragalin R=glucose
XI : trifolin R=galactose
XII : multiflorin B R=glucose-rhamnose

Furthermore, the ^{13}C–NMR spectra of asiaticalin (I), kaempferol (II), and astragalin (X) isolated from the same plant[3] were examined. The chemical shifts for the carbon atoms of these compounds were determined on full proton noise decoupled spectra as recorded in Table I. Asiaticalin (I), astragalin (X), trifolin (XI), and multiflorin B (XII) are all kaempferol 3-glycosides, of which multiflorin B (XII) was studied already by Yamasaki *et al.*[6] on the basis of ^{13}C–NMR spectrum. Therefore, the structure of asiaticalin (I) has been examined by means of ^{13}C–NMR in comparison with those of X, XI, and XII. Thus, the chemical shifts of all the carbon atoms of aglycone part were assigned to give a satisfactory agreement with those given by kaempferol (II). The ^{13}C–NMR signals of the carbon of sugar moiety of asiaticalin (I) showed a remarkable difference from those given by β-D-glucose

4) R.U. Lemieux, R.K. Kulling, H.J. Bernstein, W.G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).
5) J.D. Stevens, "Methods in Carbohydrate Chemistry," Academic Press, New York and London, VI, 1976, p. 123.
6) T. Osawa and S. Akiya, *The Bulletin of Tokyo Medical and Dental University*, **8**, 299 (1961).
7) K. Yamasaki, R. Kasai, Y. Sasaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi, and I. Nishioka, *Tetrahedron Lett.*, **1977**, 1231.

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moiety of astragalin (X). The ^{13}C-NMR spectrum of allose is being studied referring other aldohexopyranoses.

Table I. Carbon-13 Chemical Shifts in DMSO-d_6

Carbon	Aglycone moiety				Carbon	Sugar moiety	
	I	X	XI	II		I	X
2	156.5	159.3	157.3	146.1	1″	99.9	100.7
3	133.7	132.7	134.4	135.5	2″	71.6	74.2
4	177.6	176.7	177.6	175.7	3″	71.6	77.3
5	156.5	155.8	156.5	156.0	4″	67.2	69.8
6	98.9	98.4	98.9	98.2	5″	75.1	76.4
7	164.3	163.4	164.3	163.8	6″	61.3	60.8
8	93.8	93.4	93.9	93.4			
9	161.3	160.6	161.3	160.5			
10	104.1	103.7	104.2	102.9			
1′	121.0	120.6	120.5	121.6			
2′	131.1	130.5	130.7	129.3			
3′	115.3	114.8	115.5	115.3			
4′	160.0	160.6	160.0	159.0			
5′	115.3	114.8	115.5	115.3			
6′	131.1	130.5	130.7	129.3			

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and were uncorrected. UV spectra were recorded on a Hitachi 356 model in MeOH. IR spectra were recorded on a Hitachi grating 215 Infrared spectrophotometer as KBr discs. ^1H-NMR spectra were taken on a JEOL PS-100 spectrometer and ^{13}C-NMR spectra were on JEOL FX-60 spectrometer recorded in combination with a JEC-6 spectrum computer. The chemical shifts were expressed in ppm downfield from tetramethylsilan (TMS) as internal standard and coupling constants (J) were expressed in Hz. Abbreviations: s=singlet, d=doublet, t=triplet, m=multiplet.

Isolation of Asiaticalin (I)——The young trophophyll of *Osmunda asiatica* Ohwi (Osmundaceae) (15 kg) collected at Kusatsu, Gunmma Pref. and at Nagai, Yamagata Pref. in May was extracted 3 times by refluxing with MeOH for 8 hr (for each extraction). The combined MeOH solution mixed with diatomaceous earth was extracted with *n*-hexane, and then with $(CH_3)_2CO$ to yield a MeOH-soluble extract, which was concentrated to give a residue (200 g). The residue was chromatographed over silica gel to obtain a fraction (5.5 g) which gave a positive magnesium-hydrochloric acid test, and the fraction was further chromatographed on silica gel. Elution with a *n*-hexane–$(CH_3)_2CO$ mixture, then with $(CH_3)_2CO$ and crystallization from MeOH furnished asiaticalin (I), yellow needles (0.4 g), mp 182—184°, $[\alpha]_D^{20}$ slightly positive (MeOH). *Anal.* Calcd. for $C_{21}H_{20}O_{11}$: C, 56.25; H, 4.50. Found: C, 56.06; H, 4.52. UV λ_{max}^{MeOH} nm: 267, 352; UV $\lambda_{max}^{MeOH+NaOMe}$ nm: 275, 326, 402; UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 274, 301, 352, 398; UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 276, 303, 347, 400; UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 275, 305 (shoulder), 372; UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_4}$ nm: 266, 300 (shoulder), 353. IR ν_{max}^{KBr} cm^{-1}: 3401 (OH), 1656 (Ar–CO), 1606, 1504 (aromatic ring). MS: *m/e* 286 (base peak). ^1H-NMR (DMSO-d_6): 3.2—3.9 (6H, m, $C_{(2'')}\underline{H}$, $C_{(3'')}\underline{H}$, $C_{(4'')}\underline{H}$, $C_{(5'')}\underline{H}$, $C_{(6'')}\underline{H}_2$), 3.9—5.1 (4H, m, $C_{(2'')}O\underline{H}$, $C_{(3'')}O\underline{H}$, $C_{(4'')}O\underline{H}$, $C_{(6'')}O\underline{H}$), 5.68 (1H, d, (J=8.0), $C_{(1'')}\underline{H}$), 6.12 (1H, d, (J=2.0), $C_{(6)}\underline{H}$), 6.42 (1H, d, (J=2.0), $C_{(8)}\underline{H}$), 6.86 (2H, d, (J=9.0), $C_{(3')}\underline{H}$ and $C_{(5')}\underline{H}$), 8.08 (2H, d, (J=9.0), $C_{(2')}\underline{H}$ and $C_{(6')}\underline{H}$). ^{13}C-NMR (DMSO-d_6): shown in Table I. $FeCl_3$ reaction: dark green, Mg–HCl reaction: purple.

Acid Hydrolysis of Asiaticalin (I)——A solution of I (100 mg) in 2% H_2SO_4 (4 ml) was heated at 60° for 1 hr. After cooling the precipitates (60 mg) thus obtained were purified by silica gel chromatography and recrystallization from MeOH to give aglycone (II), yellow needles (48 mg), mp 275—277°. IR ν_{max}^{KBr} cm^{-1}: 3165 (OH), 1658 (Ar–CO), 1602, 1570, 1502 (aromatic ring). II was identified with an authentic sample of kaempferol by comparisons of IR spectrum and mixed mp.

The aqueous supernatant was neutralized with Amberlite IR-4B and evaporated *in vacuo* to dryness. The residue (III) was subjected to i) PPC (Toyo Filter Paper No. 51) developing with BuOH–Py–H_2O (6: 4: 3) and detected with aniline hydrogen phthalate: allose (Rf=0.53) and III (Rf=0.53), ii) TLC (Merck DC-Alufolin Cellulose F_{254}) and detected with aniline hydrogen phthalate: allose (Rf=0.28) and III (Rf=0.29), and iii) Sugar Analyzer (JEOL JLC-6AH, borate buffer pH 7.5, JEOL resin LC-R-3, absorbance at 570 nm and 425 nm). Sugars were determined with the orcinol-H_2SO_4 method: alose (205 min) and III (205 min) The Sugar (III) was identified with an authentic sample of β-D-allose.

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Methylation of Asiaticalin (I) with Diazomethane——A solution of I (150 mg) in MeOH (10 ml) was treated with the excess of ethereal CH_2N_2 at 0° for 5 days, and evaporated to dryness to give a crude product. The crude product was purified by silica gel chromatography and recrystallization from MeOH to yield three corresponding methyl ethers (VII (50 mg), VIII (25 mg), and IX (40 mg)).

7-O-Methyl asiaticalin (IV), pale yellow needles, mp 233—235°. UV λ_{max}^{MeOH} nm: 267, 352; UV $\lambda_{max}^{MeOH+NaOMe}$ nm: 274, 392; UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 274, 304, 353, 400; UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 276, 303, 348, 398; UV $\lambda_{max}^{MeOH+NaOAc}$ mn: 267, 299 (shoulder), 354; UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_4}$ nm: 266, 300 (shoulder), 352. IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1648 (Ar–CO); 1558 (aromatic ring). MS: *m/e* 300 (base peak). ^{1}H-NMR (DMSO-d_6): 3.2—3.7 (6H, m, $C_{(2'')}\underline{H}$, $C_{(3'')}\underline{H}$, $C_{(4'')}\underline{H}$, $C_{(5'')}\underline{H}$, $C_{(6'')}\underline{H}_2$), 3.48 (3H, s, –O–$COC\underline{H}_3$), 4.14 (1H, t, (J=4.5), $C_{(6'')}O\underline{H}$), 4.56 (1H, d, (J=6.5), 4.92 (1H, d, (J=4.0), 5.06 (1H d, (J=6.5), $C_{(2'')}O\underline{H}$ or $C_{(3'')}O\underline{H}$ or $C_{(4'')}O\underline{H}$), 5.68 (1H, d, (J=8.0), $C_{(1'')}\underline{H}$), 6.36 (1H, d, (J=2.0), $C_{(6)}\underline{H}$), 6.72 (1H, d, (J=2.0), $C_{(8)}\underline{H}$), 6.88 (2H, d, (J=9.0), $C_{(3')}\underline{H}$ and $C_{(5')}\underline{H}$), 8.10 (2H, d, (J=9.0), $C_{(2')}\underline{H}$ and $C_{(6')}\underline{H}$).

4′,7-O-Dimethyl asiaticalin (V), pale yellow plates, mp 230—231°. UV λ_{max}^{MeOH} nm: 266, 344; UV $\lambda_{max}^{MeOH+NaOMe}$ nm: 282, 376; UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 274, 303, 348, 394; UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 276, 301, 343, 394; UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 266, 344; UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_4}$ nm: 266, 344. IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1660 (Ar–CO), 1599, 1501 (aromatic ring). MS: *m/e* 314 (base peak). ^{1}H-NMR (DMSO-d_6): 3.2—3.7 (6H, m, $C_{(2'')}\underline{H}$, $C_{(3'')}\underline{H}$, $C_{(4'')}\underline{H}$, $C_{(5'')}\underline{H}$, $C_{(6'')}\underline{H}_2$), 3.78 (6H, s, –O–$COC\underline{H}_3\times2$), 4.12 (1H, t, ($J$=4.5), $C_{(6'')}O\underline{H}$), 4.52 (1H, d, (J=6.5), 4.86 (1H, d, (J=4.0), 5.00 (1H,d, (J=6.5), $C_{(2'')}O\underline{H}$ or $C_{(3'')}O\underline{H}$ or $C_{(4'')}O\underline{H}$), 5.66 (1H, d, (J=8.0), $C_{(1'')}\underline{H}$, 6.34 (1H, d, (J=2.0), $C_{(6)}\underline{H}$), 6.70 (1H, d, (J=2.0), $C_{(8)}\underline{H}$), 7.04 (2H, d, (J=9.0), $C_{(3')}\underline{H}$ and $C_{(5')}\underline{H}$), 8.02 (2H, d, (J=9.0), $C_{(2')}\underline{H}$ and $C_{(6')}\underline{H}$).

4′,5,7-O-Trimethyl asiaticalin (VI), pale yellow needles, mp 212—214.5°, UV λ_{max}^{MeOH} nm: 262, 335; UV $\lambda_{max}^{MeOH+NaOMe}$ nm: 263, 335; UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 267, 301, 334, 416; UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 265, 300, 330, 414; UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 262, 335; UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_4}$ nm: 262, 334. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1628 (Ar–CO), 1598, 1508 (aromatic ring). MS: *m/e* 328 (base peak). ^{1}H–NMR (DMSO-d_6): 3.2—3.6 (6H, m, $C_{(2'')}\underline{H}$, $C_{(3'')}\underline{H}$, $C_{(4'')}\underline{H}$, $C_{(5'')}\underline{H}$, $C_{(6'')}\underline{H}_2$), 3.80 (9H, s, –O–$COC\underline{H}_3\times3$), 4.23 (1H, t, ($J$=4.5), $C_{(6'')}O\underline{H}$), 4.64 (1H, d, J=6.5), 4.98 (1H, d, (J=4.0), 5.62 (1H, d, (J=4.0), $C_{(2'')}O\underline{H}$ or $C_{(3'')}O\underline{H}$ or $C_{(4'')}O\underline{H}$), 5.44 (1H, d, (J=8.0), $C_{(1'')}\underline{H}$), 6.50 (1H, d, (J=2.0), $C_{(6)}\underline{H}$), 6.78 (1H, d, (J=2.0), $C_{(8)}\underline{H}$), 7.06 (2H, d, (J=9.0), $C_{(3')}\underline{H}$ and $C_{(5')}\underline{H}$), 8.16 (2H, d, (J=9.0), $C_{(2')}\underline{H}$ and $C_{(6')}\underline{H}$).

Acid Hydrolysis of Methyl Ethers of Asiaticalin——Each of the methyl ether (IV, V, and VI) (20 mg) was heated with 2% H_2SO_4 (2 ml) at 60 ° for 1 hr. The mixture was worked up in the usual way, and each product was crystallized from MeOH to give the respective product. Pale yellow needles (VII), mp 193—195°. Pale yellow needles (VIII), mp 170—175°. Pale yellow needles (IX), mp 150—152°. VII, VIII, and IX were identified by a mixed fusion with authentic sample of 7-O-methyl kaempferol, 4′,7-O-dimethyl kaempferol, and 4′,5,7-trimethyl kaempferol, respectively.

Acknowledgement The authors are grateful to Dr. T.D. Stevens for a gift of β-D-allose. We are indebted to Assistant Professor M. Kobayashi, Meiji College of Pharmacy, to Mr. M. Higuchi, Master of Yamagata Branch, Nichiei Co., Ltd., and to Mr. H. Okuyama, Nishigo Elementary School for supplying plant material. Thanks are also due to the members of Analytical Laboratory of Meiji College of Pharmacy for elementary analysis and IR, MS, ^{1}H-NMR, and ^{13}C-NMR spectrometry.

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