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Flavonol Glycosides in *Epimedium* Species

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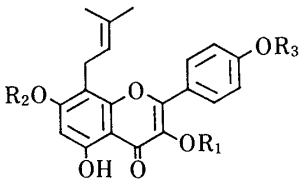
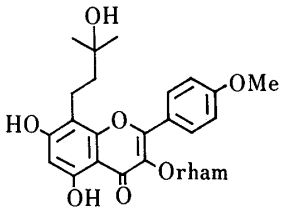
Twenty peaks due to flavonol glycosides of *Epimedium* species were identified by high-performance liquid chromatography. The structural alterations of the glycosides caused by physical factors such as heat, light and oxygen were investigated as a measure of the quality of the plant.

Keywords——flavonol glycoside; *Epimedium* species; Berberidaceae; flavonol glycoside stability; HPLC

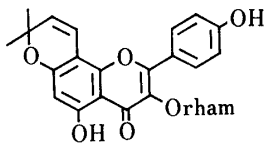
Recently the constituents of *Epimedium* species have been examined, and many new flavonol glycosides have been isolated: epimedosides A—E from the roots and rhizomes of *E. grandiflorum*,[1] icaritin-3-*O*-L-α-rhamnoside[2] and sagittatosides A—C[3] from the aerial parts of *E. sagittatum*, epimedins A—C from the herbs of *E. koreanum*,[4] and ikarisosides A—F



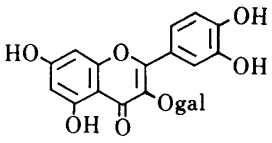
	R_1	R_2	R_3	
1	rham^2—glc	glc	Me	(epimedin A)
2	rham^2—xyl	glc	Me	(epimedin B)
3	rham^2—rham	glc	Me	(epimedin C)
4	rham	glc	Me	(icariin)
5	rham^2—glc	H	Me	(sagittatoside A)
6	rham^2—xyl	H	Me	(sagittatoside B)
7	rham^2—glc, Ac^3	H	Me	(sagittatoside C)
8	H	glc	Me	(icarisid I)
9	rham	H	Me	(icarisid II)
10	H	H	Me	(anhydroicaritin)
11	rham^2—glc	glc	H	(ikarisoside C or diphylloside A)
12	rham^2—xyl	glc	H	(epimedoside E)
13	rham^2—rham	glc	H	(diphylloside B)
14	rham	glc	H	(epimedoside A)
15	rham^2—glc	H	H	(ikarisoside B)
16	rham^2—xyl	H	H	(ikarisoside F)
17	rham	H	H	(ikarisoside A)
18	rham^4—Ac	H	H	(ikarisoside D)
19	H	H	H	(8-isoprenylkaempferol)



20



21
(ikarisoside E)



22
(hyperin)

Chart 1

from the roots of *E. grandiflorum* and *E. sempervirens*,[5] and diphyllosides A and B from the roots and rhizomes of *E. diphyllum*,[6] in addition to well-known glycosides of icariin and icarisids I and II. As a general characteristic of the glycosides in *Epimedium* species, the aglycone moiety has a γ,γ-dimethylallyl group at C-8 of kaempferol (3,5,7,4′-tetrahydroxyflavone); the C-4′ position of it is sometimes methylated to furnish anhydroicaritin, and the γ,γ-dimethylallyl moiety is occasionally hydrated at the terminal position to give icaritin. As regards the sugar moiety, a β-D-glucose is sometimes linked at C-7, an α-L-rhamnose is always linked at C-3 as an *endo*-sugar, and another α-L-rhamnose, β-D-xylose or β-D-glucose is attached to the rhamnose at its C-2 position as the terminal sugar. The flavonol glycosides of *Epimedium* species so far reported are shown in Chart 1. The present paper deals with the identification of 20 peaks of flavonol glycosides detected by high-performance liquid chromatography (HPLC) and with the alteration of the glycosides caused by physical factors such as heat, light and oxygen.

Results and Discussion

Identification of the Flavonol Glycosides

Twenty peaks detectable by HPLC were identified by comparison with authentic specimens (**1**—**22**). Aglycones **10** and **19** could not be detected with this solvent system. The results are shown in Fig. 1. The chromatogram was divided into three parts on the basis of retention times; the first one (t_R 12—15 min) (indicated hereafter as P-1) consisted of the glycosides of kaempferol with a γ,γ-dimethylally moiety at C-8 and a glucose at C-7 (**11**—**14**), the second one (t_R 20—24 min) (P-2) contained the glycosides of anhydroicaritin (**10**) with a glucose residue at C-7 (**1**—**4**), and the third one (t_R 35—48 min) (P-3) was composed anhydroicaritin glycosides (**5**—**7**) and 8-γ,γ-dimethylallylkaempferol glycosides (**15**—**18** and **21**), both of which lack a glucose at C-7. Glycosides **20** and **22** appeared apart from P-1, -2 and -3 at t_R 27 and 3.3 min, respectively.

The chromatogram of the 70% methanol extract of the aerial parts of *E. diphyllum* is showed in Fig. 2A. The extract did not contain **8**, **18** or **21**, but showed three unidentified components marked by closed circles in Fig. 2B.

Alteration of Flavonol Glycosides by Physical Factors

When the extract was boiled under reflux, the components in the extract altered after 30 h as shown in Fig. 2B. Generally flavonol glycosides in P-1 diminished in quantity, whereas

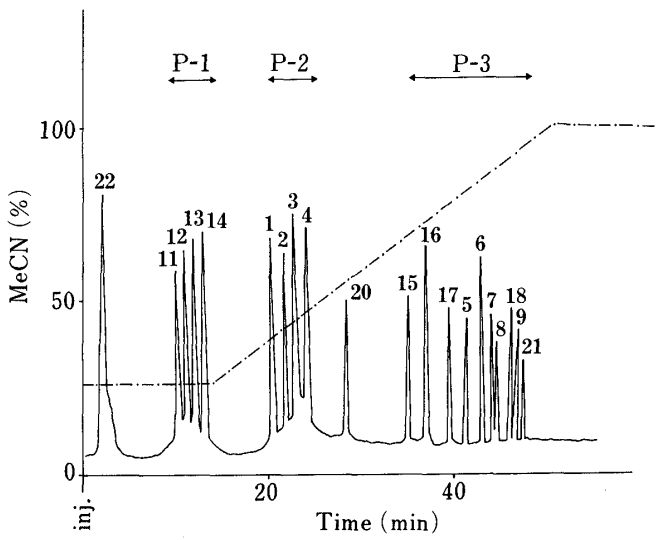


Fig. 1. High-Performance Liquid Chromatogram of the Flavonol Glycosides in *Epimedium* Species

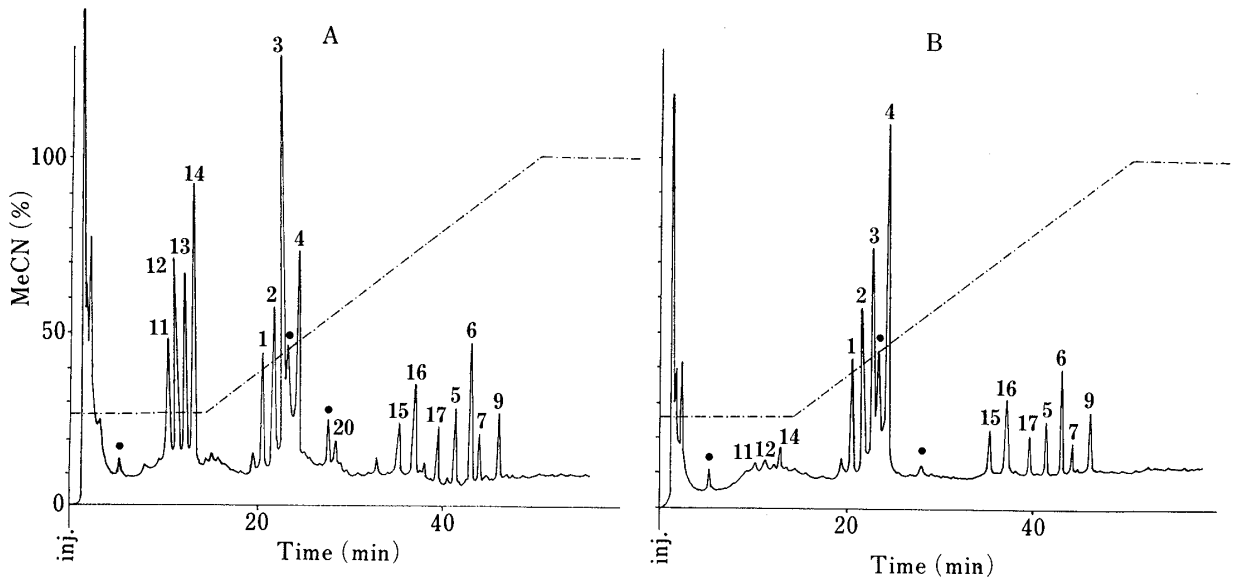


Fig. 2. HPLC Profile of the 70% Ethanol Extract of *E. diphyllum* (A) and Alteration of the Components by Boiling for 30 h (B)

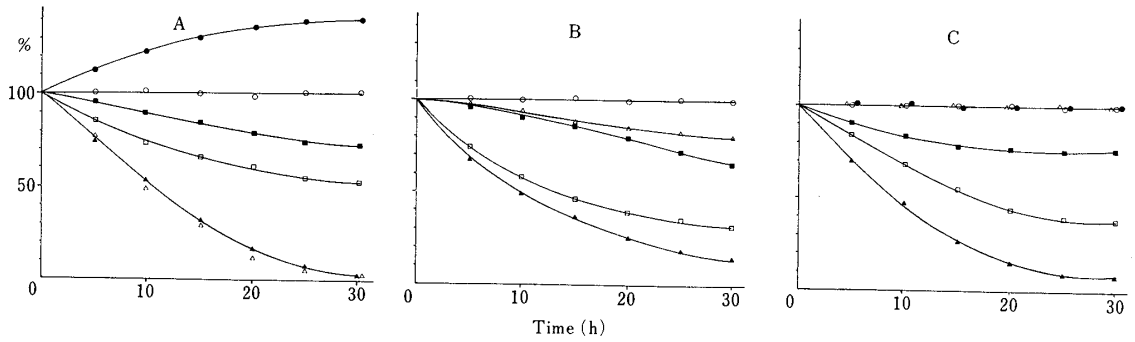


Fig. 3. Alteration of the Flavonol Glycosides in *Epimedium* Species Caused by Heat (A), Light (B) and Oxygen (C)

—○—, **1**; —■—, **3**; —●—, **4**; —▲—, **14**; —□—, **16**; —△—, **20**.

icariin (**4**) increased markedly. To investigate the decomposition of components and the structural alterations caused by physical factors, more precise experiments were carried out.

The extract was refluxed under a nitrogen atmosphere with shading in order to determine the stability of the glycosides to heat alone. The results are shown in Fig. 3A. An increase in **4** can be explained as follows; a terminal sugar at C-3 of epimedins A—C (**1**—**3**) is liable to be cleaved by heat to produce **4**. Compound **3** is most likely to release a terminal glucose because the decrease in **3** is just equal to the increase in **4**. Under the above conditions, refluxing of **3** in pure form gave **4**. Sagittatosides (**5**—**7**) decreased in quantity by 30% after 30 h. In this case, no increasing peak could be observed, which indicated that a part of the glycosides was directly hydrolyzed to yield anhydroicaritin (**10**). A decrease in ikarisosides F and A (**16** and **17**) by 80% could be explained similarly, resulting in formation of **19** by hydrolysis. The most drastic decreases in quantity were observed in **20** and **14** (only **14** is shown in Fig. 3A; glycosides **11**—**13** showed similar behavior). Glycoside **20** has two possible degradation pathways; one is cleavage of a rhamnose to yield the corresponding aglycone, and the other is an alteration of the $CH_2CH_2C(OH)(CH_3)_2$ moiety into anhydro-type to increase icarisid II (**9**). If a terminal sugar was merely hydrolyzed by heat in the cases of **11**—**13**, they would give the same glycoside **14** (epimedoside A). But no increase in **14** was actually observed, which indicated that **11**—**13**

were rapidly degraded to their aglycone without passing through **14**. The flavonol glycosides in *Epimedium* lacking a glucose at C-7 and a methyl fuction at C-4′ are deduced to be liable to decompose directly into their aglycone on heating.

The flavonol glycosides belonging to P-2 (**1** is shown in Fig. 3A as a representative) were stable to light, and were not decomposed at all under the conditions used. On the other hand, the glycosides in P-1 decreased rapidly in amount by 60% for **16**, and by 80% for **14** (including **11**, **12** and **13**). They commonly lack a glucose at C-7. Hence, it is supposed that the glycosides form a pyran ring between a hydroxy group at C-7 and the γ,γ-dimethylallyl moiety at C-8. As in the case of heat, light accelerated the degradation of the glycosides lacking a glucose at C-7.

Oxygen also affected the quantities of flavonol glycosides as shown Fig. 3C. As no increasing peaks could be observed, the glycosides were changed into their corresponding aglycones by cleavage of all sugars.

In the present study, the apparent amount of icariin does not always reflect the quality of *Epimedium* species because the amount determined depends on the extraction method. Furthermore, the glycosides in P-1, which are major components in the roots and rhizomes, are not easy to determine because they are too unstable to physical factors. Since ancient times, the materials of *Epimedium* species have been extracted with alcohol for medicinal use so as not to decompose the intact flavonol glycosides, and this is consistent with the present results concerning their instability to physical factors.

Experimental

The leaves of *Epimedium diphyllum* were collected at Miyazaki prefecture, Japan, in November 1986. A sample has been deposited in the Herbarium of Gifu Pharmaceutical University. The authentic specimens used for HPLC were isolated by us (**1**—**14**, **19**, **20** and **22**)[2,3,6] or donated (**15**—**18** and **21**).[5]

HPLC Equipment——Liquid chromatograph: Shimadzu LC-6A. Detector: Shimadzu UV spectrometric detector SPD 6A. Injector: Rheodyne 7215. Recorder: Shimadzu R-11. Conditions: column, Cosmosil $_5C_{18}$ (Nakarai Chemicals Ltd.) 250 × 4 mm i.d.; flow rate, 1.2 ml/min; detection, ultraviolet (UV) 272 nm; chart speed, 2.5 mm/min; mobile phase, gradient program shown as a broken line in Fig. 2.

Apparatus for Examining Stability of Flavonol Glycosides——A 70% methanol extract (300 ml) of the aerial parts (4.0 g) of *E. diphyllum* was prepared by extraction on an ultrasonic water bath at room temperature for 30 min. An aliquot (50 ml) was used for each experiment. (a) An extract in a three-necked flask (200 ml) fitted with a reflux condenser and nitrogen inlet tube was covered with aluminium foil, and then boiled at 100 °C. Periodically, 1 ml of the extract was taken out as sample for a HPLC. (b) An extract in a photochemical apparatus (Riko Kagaku Ltd.) equipped with a 100 W high-pressure mercury lamp was irradiated under a nitrogen atmosphere with stirring. (c) Air (300 ml/min) was passed through an extract at room temperature in a dark room. After filtration through a membrane (0.45 μm), each sample (10 μl) was injected into the HPLC column.

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