BIOACTIVE COMPOUNDS OF THE FLORA OF BELARUS. 3.* *Gymnocarpium dryopteris*, A KAEMPFEROL SOURCE

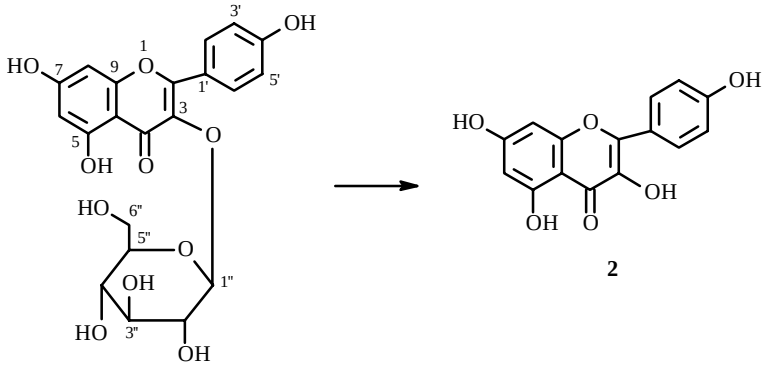
N. V. Kovganko, Zh. N. Kashkan, and S. N. Krivenok

UDC 547.814.5

*Kaempferol (**2**) was isolated from the aerial part of the oak fern* Gymnocarpium dryopteris *by extraction and subsequent acid hydrolysis of the glycoside mixture.*

Key words: kaempferol, fern, *Gymnocarpium dryopteris.*

We previously isolated from the oak fern *Gymnocarpium dryopteris* (Aspidiaceae) the flavonolic glycoside astragalin **1**, which is a rather effective protector of the enzyme catalase from ultrasonic inactivation [2]. The structural formula shows that astragalin has the chemical structure kaempferol-3-O-β-D-glucoside. Therefore, it seemed interesting to use oak fern as raw material to produce the aglycon of astragalin, the flavonol kaempferol **2**. Kaempferol has antiviral [3], antioxidant [4], and antiprotozoic [5] activities and is an inhibitor of adenosine deaminase [6]. It should also be noted that it can be used in cosmetic formulations [7] and as one of the components of food additives [8].



1 **2**

Several methods are known for producing kaempferol from several plants in which it occurs either in the free state or as glycosides [9-12]. In particular, the isolation of this flavonol from flowers of *Rosa damascena* [9], *Trifolium alexendrium* [10], heartwood of *Podocarpus spicatus* [11], and the aerial part of *Vinca erecta* [12] has been described.

Kaempferol was produced from oak fern first by hydrolysis of astragalin **1**. Thus, treatment of **1** with dilute aqueous HCl and boiling produced aglycon **2** in high yield. The structure was unambiguously found from spectral data. The most convincing proof was obtained by comparing its PMR spectrum with that of the starting astragalin (Table 1). The position and shape of the signals for all aromatic protons in the PMR spectra of **1** and **2** are practically identical. However, the spectrum of **2** lacks signals for the glucose protons, the presence of which is characteristic for the spectrum of astragalin.

It should also be noted that the principal features of the PMR spectrum of **2** obtained by us agree well with those for the spectrum of this same compound [13].

*For Parts 1 and 2, see Ref. 1 and 2.

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Minsk, ul. Akad. Kuprevicha, 5/2, e-mail: kovganko@iboch.bas-net.by. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 16-17, January-February, 2004. Original article submitted November 11, 2003.

 0009-3130/04/4001-0016

TABLE 1. PMR Spectra (δ, ppm) of Compound **1** and **2**

Proton	**1**		**2**
H-6	6.79 s	6.25 d (J = 2 Hz)	6.16 d (J =1.5 Hz)
H-8	6.79 s	6.49 d (J = 2 Hz)	6.375 d (J = 1.5 Hz)
H-3′, H-5′	7.34*	6.92 d (J = 8.5 Hz)	6.90 d (J = 8.5 Hz)
H-2′, H-6′	8.49 d (J = 8.5 Hz)	8.07 d (J = 8.5 Hz)	8.075 d (J = 8.5 Hz)
H-1″	6.40 d (J = 6.5 Hz)	5.49 d (J = 6.5 Hz)	
5-OH		12.53 s**	
7-OH		10.87 s**	
4′-OH		10.16 s**	
Solvent	C_5D_5N	$(CD_3)_2SO$	CD_3OD

*Partially overlapped by solvent signal.

**Signal disappears upon D-exchange with D_2O.

It was found [1] that astragalin **1**, although the principal flavonoid glycoside of oak fern, is not the only one. Our several attempts to isolate the pure minor glycosides and establish their structures were unsuccessful. This was due mainly to their low thermal and chemical stabilities. However, we were able to determine from PMR spectra that the major part of these compounds is kaempferol glycosides. On this basis we hypothesized that kaempferol from *G. dryopteris* can be produced by a simpler method and in greater yield than noted above. For this, acid hydrolysis of the total glycosides without isolating them pure is necessary in one of the early steps. In fact, our observations showed that extraction of the aerial part of oak fern by ethanol, concentration of the total glycosides in *n*-butanol solution, and hydrolysis by dilute HCl can produce kaempferol **2** in 0.38% yield calculated per air-dried raw material.

Thus, in our opinion oak fern (*G. dryopteris*) is a convenient plant raw material for isolating both astragalin (**1**) and kaempferol (**2**).

EXPERIMENTAL

Melting points were determined on a Kofler block. UV spectra of ethanol solutions were recorded on a Specord M-400 instrument. PMR spectra were obtained on a Bruker AC-200 NMR spectrometer at working frequency 200 MHz. Chemical shifts are given relative to TMS as an internal standard.

Hydrolysis of 1. Astragalin (**1**, 0.100 g) (isolated from *G. dryopteris* by the literature method [1]) was treated with HCl (30 mL, 6%). The solution was boiled for 1 h with constant stirring, cooled to room temperature, and extracted with ethylacetate (3 × 5 mL). The combined extracts were evaporated and dried in vacuum to give **2**, 0.0584 g, 91.1%, mp 280-290°C (dec.), lit. mp 280-283°C (dec.) [9], 275-277°C [10]. UV spectrum (λ_{max}, nm, EtOH): 267, 367.

Production of 2 from *G. dryopteris*. The dried and finely ground aerial part of oak fern (*G. dryopteris*) (69 g) that was collected in June 2002 near Minsk was extracted with ethanol (3 × 500 mL) at room temperature. The solvent was removed in vacuum. The solid was dissolved in ethanol (30%, 200 mL) and extracted three times with petroleum ether in portions of 200, 50, and 50 mL. The ethanol was evaporated in vacuum from the aqueous ethanol layer. The solid was extracted three times with *n*-butanol in portions of 50, 30, and 25 mL. Removal of solvent in vacuum from the combined butanol extract afforded a solid (1.88 g) that was dissolved in HCl (50 mL, 6%), boiled with stirring for 1 h, cooled to room temperature, and extracted with ethylacetate (3 × 25 mL). The combined extract was evaporated in vacuum. The solid was chromatographed over a column of silica gel with elution by ethylacetate to afford **2** (0.262 g), which was identical to that obtained above. Yield 0.38% calculated for air-dried raw material.

REFERENCES

1. N. V. Kovganko, Zh. N. Kashkan, and S. N. Krivenok, *Khim. Prir. Soedin.*, 274 (2002).
2. N. V. Kovganko, Zh. N. Kashkan, S. N. Krivenok, M. V. Potapovich, A. N. Eremin, and D. I. Metelitsa, *Khim. Prir. Soedin.*, 62 (2003).
3. D. Mitrocotsa, S. Mitaku, S. Axarlis, C. Harvala, and M. Malamas, *Planta Med.*, **66**, No. 4, 377 (2000).
4. R. A. Ronzio, D. N. Muanza, and W. S. Sparks, U.S. Pat. No. 5,762,936, June, 9, 1998.
5. F. Calzada, M. Meckes, and R. Cedillo-Rivera, *Planta Med.*, **65**, No. 1, 78 (1999).
6. M. F. Melzig, *Planta Med.*, **62**, No. 1, 20 (1996).
7. G. Lanzendorfer, F. Stab, and S. Untiedt, U.S. Pat. No. 6,423,747, July, 23, 2002.
8. A. N. Howard, S. V. Nigdikar, J. Rajput-Williams, and N. R. Williams, U.S. Pat. No. 6,099,854, Aug., 8, 2000.
9. G. N. Zemtsova, *Khim. Prir. Soedin.*, 783 (1982).
10. V. K. Saxena and A. K. Jain, *J. Indian Chem. Soc.*, **63**, No. 9, 854 (1986).
11. L. H. Briggs, R. C. Cambie, and J. L. Hoare, *Tetrahedron*, **7**, No. 3/4, 262 (1959).
12. V. I. Akhmedzhanova, *Khim. Prir. Soedin.*, 638 (1986).
13. A. Grouiller and H. Pacheco, *Bull. Soc. Chim. Fr.*, No. 6, 1938 (1967).