Chemistry of Natural Compounds, Vol. 38, No. 4, 2002

BIOACTIVE SUBSTANCES OF THE FLORA OF BELARUS.

1. ASTRAGALIN FROM *Gymnocarpium dryopteris*

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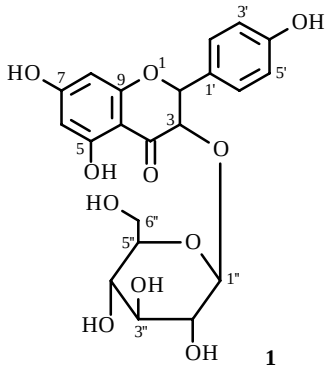
*The flavonol glycoside astragalin **1** was isolated from the aerial part of* Gymnocarpium dryopteris *in 0.70% yield. Its structure was determined using spectral data.*

Key words: *Gymnocarpium dryopteris*, astragalin.

Gymnocarpium dryopteris (synonym *Dryopteris linnaeata*) (tripinnate oak fern or Linneus fern) is rather prolific in forests of Belarus [1]. It is classified botanically as a member of the *Dryopteris* Adans. genus, a wood fern (Aspidiaceae). The phytochemistry of this species is poorly studied. It is known only that it contains significant quantities (8-12% in rhizomes) of tanning agents [2], gibberellins [3], and fluoroglucinol derivatives [4]. Ferns closely related to this species have been studied in more detail. For example, rhizomes of *Dryopteris filixmass* (L.) Schott contain a large amount of fluoroglucinol derivatives that are used in medicine under the name filicin as an effective antihelminthic [5, 6].



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Phytoecdysteroids occur in large amounts in some fern-like plants [7]. Analysis by TLC detected in the CH_3OH extract of the aerial part of tripinnate oak fern a high content of substances absorbing in the UV region that have chromatographic properties similar to those of 20-hydroxyecdysone. A scheme that is most often used for phytoecdysteroids was used to isolate and purify them. Thus, a pale yellow substance was isolated from the CH_3OH extract. According to spectral data, it was not an ecdysteroid. In particular, the IR spectrum of this compound is consistent with the presence of several hydroxyls, a carbonyl conjugated to a double bond, and aromatic rings. However, the C–H stretching vibrations at 2800-3000 cm^{-1} in the spectrum are weak. The presence of three bands at 353, 300, and 267 nm in the UV spectrum indicate that this compound is a plant phenol. According to the literature [8], spectra of flavonoids appear like this. The PMR of **1** in deuteropyridine (Table 1) exhibits signals of several aromatic protons that are located, judging from their chemical shifts and multiplicities, next to hydroxyl groups. Furthermore, the PMR has a doublet for an anomeric proton at δ 6.40 ppm. Therefore, this molecule contains a carbohydrate unit. The ^{13}C NMR spectrum of this compound (Table 2) in deuteropyridine can, first, determine its structure as a flavonoid and, second, establish that its structure includes a monosaccharide unit. Comparison of the chemical shifts of the corresponding atoms in the ^{13}C NMR spectrum of this glycoside with those in the literature [9] for the spectra of certain flavonoids led us to the conclusion that the aglycone of the isolated glycoside is kaempferol. Its spectrum indicates that the

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 0009-3130/02/3804-0328$27.00

TABLE 1. PMR Spectra (δ, ppm, J/Hz) of Astragalin **1**

Proton	δ	δ	δ [10]
H-6	6.79s	6.25 d (2)	6.21 d (2)
H-8	6.79s	6.49 d (2)	6.44 d (2)
H-3′, H-5′	7.34*	6.92 d (8.5)	6.88 d (8.8)
H-2′, H-6′	8.49 d (8.8)	8.07 d (8.5)	8.04 d (8.8)
H-1′′	6/40 d (6.5)	5.49 d (6.5)	5.45 d (7.4)
5-OH		12.53 s**	
7-OH		10.87 s**	
4′-OH		10.16 s **	
Solvent	C_5D_5N	$(CD_3)_2SO$	$(CD_3)_2SO$

*Partially overlaps the solvent signal.
**Signal disappears upon D-exchange in D_2O.

TABLE 2. ^{13}C NMR Spectra (δ, ppm) of Astragalin **1**

Atom	δ	δ	δ [10]
C-2	162.8	161.1	159.9
C-3	-*	133.1	133.1
C-4	178.7	177.3	177.4
C-5	157.3	156.1	155.9
C-6	99.8	98.7	98.6
C-7	165.9	164.4	164.1
C-8	94.6	93.6	93.6
C-9	157.5	156.3	156.2
C-10	105.2	103.8	103.9
C-1′	121.9	120.8	120.8
C-2′	131.8	130.8	130.8
C-3′	116.1	115.0	115.0
C-4′	161.9	159.9	159.9
C-5′	116.1	115.0	115.0
C-6′	131.8	130.8	130.8
C-1″	103.8	100.8	100.8
C-2″	76.4	74.1	74.1
C-3″	78.5	76.3	76.3
C-4″	71.4	69.8	69.8
C-5″	79.0	77.4	77.4
C-6″	62.5	60.7	60.8
Solvent	C_5D_5N	$(CD_3)_2SO$	$(CD_3)_2SO$

*Overlaps the solvent signal.

carbohydrate part is D-glucose. The chemical shifts of C-2, C-3, and C-4 in the spectrum of **1** differ most from those of the analogous atoms in the spectrum of kaempferol [9]. Therefore, we concluded that this is a 3-O-glucoside. In fact, the PMR and ^{13}C NMR spectra of **1** in DMSO-d_6 (Tables 1 and 2, respectively) are identical to those reported by us [10] for the flavonoid astragalin with the structure kaempferol-3-O-*β*-D-glucoside. Therefore, this proves the structure of the compound isolated from tripinnate oak fern.

It should be noted that astragalin was isolated for the first time from flowers of *Astragalus sinicus* [11]. It has also been found in the ferns *Pteridium acquilinum* [12] and *Plenasium banksiifolium* [13]. Our data indicate that astragalin is the principal flavonoid in tripinnate oak fern. The structures of the minor flavonoids of this plant are under investigation.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded in KBr pellets on a UR-20 instrument in the range 700-3600 cm^{-1}. UV spectra of ethanol solutions were obtained on a Specord M-400 instrument. PMR and ^{13}C NMR spectra were obtained on a Bruker AC-200 NMR spectrometer at working frequencies 200 and 50.32 MHz, respectively. The multiplicity of signals in the ^{13}C NMR spectrum was determined from DEPT spectra. Chemical shifts are given relative to TMS internal standard.

Isolation of Astragalin (1). Dried and finely ground fronds of *Gymnocarpium dryopteris* (63.5 g) collected in July 2001 near Minsk were extracted with CH_3OH (3×500 mL) at room temperature. The solid obtained via evaporation in vacuum was dissolved in MeOH (30%, 200 mL) and washed with hexane (200 mL). The MeOH was evaporated in vacuum. The solid was extracted twice with *n*-BuOH (50 and 25 mL) and evaporated in vacuum to afford 1.73 g of solid. A part of the solid (1.50 g) was chromatographed over a silica-gel column with elution under N_2 pressure by CH_3OH with CH_2Cl_2 and then pure CH_3OH. Fractions containing compounds with R_f values close to those of 20-hydroxyecdysone according to TLC were collected to give a solution of the total flavonoid glycosides. Partial evaporation of solvent precipitated light yellow crystals that were filtered off to give astragalin (**1**, 0.18 g). Further concentration of the mother liquor isolated another 0.20 g of **1**. Overall yield, 0.70% calculated per air-dried raw material, mp 173-176°C (CH_3OH), lit. mp 177-178°C [10], 178°C [11], 208.5-210°C [13]. IR spectrum (ν, cm^{-1}): 3420 (OH), 1680 (C=O), 1620 (C=C), 1590, 1510 ($C=C_{arom}$). UV spectrum (λ_{max}, nm): 353 (ε 14,000), 300 (ε 9,000), 267 (ε 15,800).

Solvent was evaporated from the mother liquor after astragalin (**1**) was removed to give minor flavonoids (0.19 g).

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