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FLAVOLIGNANS OF *Silybum marianum* FRUIT

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*The chemical composition of fruit from spotted milkweed [*Silybum marianum *(L.) Gaertn.] cultivated in Samara district was investigated using UV and ^{1}H-NMR spectroscopies and mass spectrometry. Chemical transformations identify the flavolignans silybin, silydianin, silychristin, and 2,3-dehydrosilybin. The last was first described for spotted milkweed cultivated in Russia and CIS countries.*

Key words: *Silybum marianum*, fruit, flavolignans, hepatoprotectors.

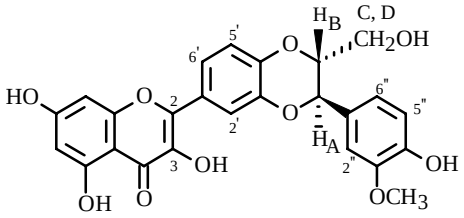
Hepatoprotectors based on fruit of spotted milkweed [*Silybum marianum* (L.) Gaertn.] are widely used in medicine. However, expensive foreign preparations (carsil, legalon, siliborum, and other) still dominate the market [1-3]. The domestic hepatoprotectors silimarum and liquid milkweed extract that are based on fruit of spotted milkweed cultivated in Samara district (Russia) have been developed and introduced [2, 3]. We have previously observed in this plant using HPLC the dominating flavolignans silybin, silydianin, and silychristin [4] of flavanonol nature [5-7].

A thorough investigation of the chemical composition of spotted milkweed fruit cultivated in Samara district using column chromatography identified four compounds of flavonoid nature (**1**-**4**).

The NMR spectrum of compound **1** lacks signals for H-2 and H-3 (doublets with J = 11 Hz at 4.5-5.1 ppm, characteristic of flavolignans **2-4** of flavanonol nature). The structure of the flavonol-lignan is confirmed by the mass spectrum with a molecular ion of mass 480. A positive test with H_2SO_4 (lemon-yellow color like for silybin) indicates that compound **1** contains a benzodioxane ring (this test is negative for silychristin).

This compound has TLC R_f values of 0.45 ($CHCl_3$—CH_3OH, 6:1) and 0.8 ($CHCl_3$—CH_3OH—H_2O, 26:14:3) and gives a bright green fluorescent spot (flavonol) upon development by $AlCl_3$.

Therefore, compound **1** is 2,3-dehydrosilybin, which was isolated previously by Indian and Japanese researchers [8, 9] and synthesized by German researchers from silybin [7].



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Flavolignans **2-4** have a flavanonol nature and are 1,4-benzodioxanes (silybin), tricyclic ketones (silydianin), and benzofurans (silychristin), respectively [1, 5-7]. Silybin (**1**) was first described by German researchers [10, 11] and then isolated by us from fruit of spotted milkweed cultivated in Samara district. It was proposed as a State standard in qualitative and quantitative analysis of the raw material and preparations of this plant [2].

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Flavolignans **3** and **4** were first isolated by us from fruit of spotted milkweed cultivated in Samara district. Their physicochemical and spectral properties are identical to silydianin and silychristin, respectively [6, 7].

Thus, the results indicate that the isolated flavolignans are silybin, silydianin, silychristin, and 2,3-dehydrosilybin. The last is first described from spotted milkweed cultivated in Russia and CIS countries.

EXPERIMENTAL

^{1}H NMR spectra were measured on a Varian—Gemini 200 instrument. UV spectra were recorded on a Specord M40 instrument in CH_3OH. Electron-impact mass spectra were measured on a Varian CH-8 instrument at ionizing-electron energies 35 and 70 eV and ion-source temperatures from 30 to 250°C.

Isolation and Separation of Flavolignans. Spotted milkweed fruit (2000 g harvest) cultivated industrially in Samara district (Sergievskii state farm) was exhaustively extracted with ethanol (80%) at a 1:7 raw-material—extractant ratio. The combined extracts were evaporated in vacua to a thick residue and were mixed with L 40/100 silica gel. The extract was dried on the silica gel and placed on a silica-gel chromatography column as a suspension in $CHCl_3$. The column was eluted by $CHCl_3$—C_2H_5OH mixtures in various ratios (100:0→80:20). The flavolignan (**2**) was isolated and purified by recrystallization from alcohol. Rechromatography on Woelm polyamide of the fractions containing other target compounds and subsequent recrystallization isolated flavolignans **1**, **3**, and **4**.

The course of the chromatographic separation was monitored using TLC on Silufol UV-254 (15×15 cm) and Sorbfil PTSKh-P-A-UV (10×10 cm) preparative plates using solvent systems $CHCl_3$—CH_3OH (6:1), $CHCl_3$—CH_3OH—H_2O (26:14:3), and $CHCl_3$—$(CH_3)_2CO$—HCO_2H (9:2:1). Compounds were detected by viewing the chromatograms in UV light of wavelengths 254 nm (chromatoscope) and 366 nm and also by visualization using $AlCl_3$ solution and freshly prepared diazobenzenesulfonic acid solution.

2,3-Dehydrosilybin (1). Yellow crystals, $C_{25}H_{20}O_{10}$ (M^+ 480), mp 253-255°C (EtOH). UV spectrum (λ_{max}): 267 and 365 nm. ^{1}H NMR spectrum [$(CD_3)_2CO$, 200 MHz, J/Hz]: 12.15 (1H, s, 5-OH), 9.74 (1H, br.s, 7-OH), 7.87 (1H, d, J = 2.1, H-2^1), 7.17 (1H, dd, J = 8.3 and J = 2.1, H-6^1), 7.8-6.9 (4H-Ar, m), 6.60 (1H, d, J = 2.1, H-8), 6.28 (1H, d, J = 2.1, H-6), 5.06 (1H, d, J = 8.06, H_A), 4.24 (1H, m, H_B), 3.88 (3H, s, CH_3O), 3.80 (1H, dd, J = 12.5 and J = 3.5, H_C), 3.55 (1H, dd, J = 12.5 and J = 4.3, H_D).

Silybin (2). White crystals, $C_{25}H_{22}O_{10}$ (M^+ 482), mp 164-166°C, $[\alpha]_D$ +10.8 (acetone). UV spectrum (λ_{max}): 289 and 325 (sh) nm. ^{1}H NMR spectrum [$(CD_3)_2CO$, 50°C, 200 MHz]: 11.70 (1H, s, 5-OH), 6.8-7.2 (6H-Ar, m), 5.96 (2H, d, J = 2, H-6.8), 5.10 (1H, d, J = 12, H-2), 5.00 (1H, d, J = 8, H_A), 4.60 (1H, d, J = 12, H-3), 4.1-4.2 (1H, m, H_B), 3.3-3.8 (2H, m, H_C and H_D), 3.85 (3H, s, CH_3O).

Silydianin (3). White crystals, $C_{25}H_{22}O_{10}$ (M^+ 482), mp 188-191°C. UV spectrum (λ_{max}): 289 and 325 (sh) nm. ^{1}H NMR spectrum [$(CD_3)_2CO$, 200 MHz, J/Hz]: 11.76 (1H, s, 5-OH), 9.78 (1H, br.s, 7-OH), 6.7-7.0 (3H-Ar, m), 6.30 (1H, m, H-6^1), 6.02 (2H, br.s, H-68), 4.92 (1H, dd, J = 12 and J = 2, H-2), 4.65 (1H, dd, J = 12 and J = 3, H-3), 4.29 (1H, dd, J = 8 and J = 3.5, H_C), 3.78 (1H, d, J = 8, H_D), 3.75 (3H, s, CH_3O), 3.66 (1H, dd, J = 4 and J = 2, H-2^1), 3.42 (1H, m, H_A), 3.28 (1H, dd, J = 6 and J = 2.5, H-5^1), 2.9 (1H, m, H_B).

Silychristin (4). Light yellow crystals, $C_{25}H_{22}O_{10}$ (M^+ 482), mp 173-175°C, $[\alpha]_D$ +207 (ethanol). UV spectrum (λ_{max}): 289 and 326 (sh) nm. ^{1}H NMR spectrum [$(CD_3)_2CO$, 200 MHz, J/Hz]: 11.86 (1H, s, 5-OH), 6.8-7.18 (6H-Ar, m), 6.0 (1H, d, J = 2, H-8), 5.96 (1H, d, J = 2, H-6), 5.60 (1H, d, J = 6.4, H_A), 5.05 (1H, d, J = 11.5, H-2), 4.65 (1H, d, J = 11.5, H-3), 4.1-4.3 (1H, m, H_B), 3.5-3.8 (2H, m, H_C and H_D), 3.82 (3H, s, CH_3O).

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