Thus, (II) had the structure of luteolin 3'-O-β-D-glucofuranoside and is a new flavone glycoside.

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IDENTIFICATION OF 2',7-DIHYDROXY-4'-METHOXYISOFLAVAN (VESTITOL) IN THE ROOTS OF ALSIKE CLOVER

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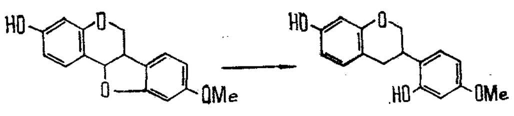
Trifolium hydribum (Alsike clover) differs from *Tr. pratense* (red clover) by a considerably greater resistance to unfavorable factors of the external environment and to fungal diseases [1]. In the search for compounds possessing antifungal activity and present in the roots of this plant — the organs subject to attack — we obtained an acetone extract and, by its separation on columns of silica gel in a benzene–acetone gradient system, we have isolated a small amount of a crystalline substance (I) with mp 156°C (chloroform) having the composition $C_{16}H_{16}O_4$.

The NMR spectrum of this substance taken in deuteroacetone solution contained a three-proton singlet of an aromatic methoxy group at 3.85 ppm, with two broadened one-proton singlets of OH groups at 8.06 and 8.51 ppm, and also a group of peaks of aromatic and aliphatic protons. These facts show the partial formula of the substance as $C_{15}H_{11}O(OH)_2(OMe)$. The absence from the IR spectra of the substance of the absorption of a C=O group and the presence of the characteristic system of proton signals for a $-CH_2-\overset{|}{C}H-CH_2$ group at 4.3-2.9 ppm [2] showed the isoflavan structure of the molecule. This was in agreement with the UV spectrum taken in MeOH solution: λ_{max} (nm) 206, 228, 285 (ε 45,500, 11,200, 5600).

The aromatic protons in the NMR spectrum of the substance taken in $CDCl_3$ solution formed an ABX system with the spin–spin coupling constants $J_{AX} = 8$ Hz, $J_{AB} = 3$ Hz, and $J_{BX} = 0$, which corresponds to 1,2,4-trisubstitution of both the aromatic rings of the molecule. The position of the methoxy group in it followed from the results of mass spectrometry: The spectra showed the presence of two high-intensity ions with m/e 150, having the composition $C_9H_{10}O_2$, and the m/e 137, having the composition $C_8H_9O_2$, formed from ring B of the isoflavan molecules [3] which corresponds to the presence of the methoxy group in this fragment. The choice between 2' and 4' positions for this group was made on the basis of the reductive decyclization of natural (−)-medicarpin (II) over 10% Pd/C (AcOH, 20°C). The substance formed by this reaction was completely identical (UV, NMR, and mass spectra) with compounds (I) that we had isolated.



II I

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The isoflavan isolated proved to be the levorotatory isomer with $[\alpha]_D^{20}$ −15.8° (c 1.2; MeOH), which, according to the literature [4], determines its 3R configuration. Thus, this compound is (−)-2',7-dihydroxy-4'-methoxyisoflavan (vestitol). Vestitol has been isolated previously from a number of plants. It possesses a high antifungal activity and is considered to be the phytoalexin of alfalfa leaves and of the leguminous plant *Lotus corniculatus* [5].

On separating under similar conditions an acetone extract of red clover roots we detected no fractions containing vestitol and its very similar 2'-methyl analog (sativin), which possibly in part explains its lower resistance to fungal diseases.

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CHEMICAL STUDY OF *Melampyrum elatius*

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We have studied the epigeal part of *Malampyrum elatius* Reuter, collected in the flowering period.

The air-dry raw material was extracted in the cold successively with 96% ethanol and with aqueous acetone, and the combined extracts were concentrated under reduced pressure and were investigated for their content of polyphenolic compounds. Paper chromatography revealed four compounds belonging, according to their color reaction, to the flavones and their 7-glycosides.

The concentrated residue of the extract was treated with hot water, and the aqueous extract was purified with chloroform and was then treated several times with ethyl acetate. The combined ethyl acetate extracts were dried with sodium sulfate and poured into a fivefold volume of dry chloroform. The mixture was left to stand in the refrigerator for several days, and then the intense yellow precipitate that had deposited, consisting of a mixture of two glycosides, was taken off. The substances were separated by sublimation [1], in which process substance (I) sublimed readily.

Substance (I) formed bright yellow crystals. UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 260, 340 nm. It readily underwent hydrolysis with the formation of luteolin and L-arabinose. The glycoside did not take part in the azo-coupling reaction, which shows the absence of a free hydroxyl at C_7 [2] and, consequently, it can be characterized as luteolin 7-arabinoside. Because of its lability, this substance has not been studied in more detail.

Substance (II) formed pale yellow crystals. UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 256, 350 nm. No bathochromic shift was observed in the presence of sodium acetate; on the addition of $AlCl_3$: λ_{max} 274, 328, 432 nm; $AlCl_3 + HCl$: 274, 294 sh, 387 nm; $H_3BO_3 + CH_3COONa$: 254, 372 nm. R_f 0.16 (15% CH_3COOH), 0.41 (n-butanol—CH_3COOH—H_2O (4:1:5)). Hydrolysis formed luteolin and D-glucose.

The substance was characterized as luteolin 7-glucoside.

From the aqueous ethanolic extract, after its concentration, the elimination of lipophilic impurities, and the extraction of polyphenolic compounds with butyl acetate, chromatography on a column filled with previously swollen filter paper (eluent aqueous ethanol) yield-

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