Syringaresinol diglucoside (eleutheroside D) (XV) – white acicular crystals with the composition $C_{33}H_{38}O_{13}$, mp 255-257°C (aqueous alcohol), λ_{max}^{EtOH} 234, 271 nm, $[\alpha]_D^{23}$ −6.1° (0.4; 50% ethanol).

Compound (XV) was hydrolyzed by β-glucosidase with the formation of syringaresinol (XIV) and glucose.

The results presented show that we are the first to have isolated compounds (I-IX) from this plant, while we have confirmed the presence of compounds (X-XV) [2, 3, 5]. At the same time, it must be mentioned that we did not detect certain compounds described previously for this plant: the lignan sesamin [4, 5], ethyl caffeate [5], and sinapyl alcohol [5].

The fact deserves attention that the chlorogenic acid (VIII) detected previously by the TLC method [5] and successfully isolated by us is one of the dominating components of the plant and (together with lignans and other phenylpropanoids) affects the nature of the UV absorption curve of an extract of prickly eleutherococcus (λ_{max}^{EtOH} 284 and 330 nm).

The greatest practical interest is presented by syringin (X), giving on Silufol UV 254 plates [chlf–MeOH–H_2O (26:14:3)] a bright blue coloration on treatment with 16% sulfuric acid (110°C). This method (using a standard sample of syringin) may be used for determining the authenticity of the raw material and of eleutherococcus preparations and also of those from other plants containing syringin (lilac, mistletoe).

LITERATURE CITED

1. I. V. Darymov, Ginseng, Eleutherococcus (On the Mechanism of Their Biological Action) [in Russian], Nauka, Moscow (1976).
2. Yu. S. Ovodov, R. G. Ovodova, T. F. Solov'eva, G. B. Elyakov, and N. K. Kochetkov, Khim. Prir. Soedin., No. 1, 3 (1965).
3. Yu. S. Ovodov, G. M. Frolova, L. A. Elyakova, and G. B. Elyakov, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, 11, 2065 (1965).
4. N. I. Suprunov and S. N. Dzizhenko, Khim. Prir. Soedin., No. 4, 524 (1971).
5. H. Wagner, H. Heuer, A. Abermeier, G. Tittel, and S. Bladt, Planta Med., 44, 193 (1982).

PROTOLYTIC PROPERTIES OF THE PHYTOMELANIN PIGMENTS OF Helianthus annuus

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One of the main problems of the chemistry of phytomelanin pigments (PMPs) holding back their use in medicine and the pharmacological, chemical, and food industries is the structural-group differentiation of the protogenic oxygen functional groups, without which the standardization of PMP preparations is impossible. The amounts of these functional groups and the determination of their capacity for ionization have been little studied in practice in view of the difficulties due to the statistical copolymeric structure, the polymolecularity, and the limited solubility of the pigments [1].

As an illustration of these difficulties we may give the molecular absorption spectra of the PMPs, which have unresolved structures in the UV and VIS regions of the spectrum.

Taking as an example a study of the protolytic equilibria of the H, NH_4, and Na forms of the PMPs of the husks of black seeds of Helianthus annuus in dilute (0.005-0.01%) aqueous solutions in the pH range of 3-12, we have shown the possibility of a differentiation of carboxylic and phenolic functional groups with respect to their ionization constants (pK) and their mass fractions (W). To find these parameters we used the methods of direct and

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reverse protolytometry in the variants of spectrophotometric and potentiometric titration, and conductometric titration at low and high frequencies, giving on the titration curves in the case of direct protolytometry well-defined jumps or breaks the first of which corresponds to the neutralization of carboxylic, and the second and third to that of phenolic, functional groups. In reverse protolytometry, this sequence of jumps is preceded by a jump due to the neutralization of the excess of alkali. The order of titration of the functional groups was checked independently by the methylation of the PMPs with various reagents: absolute CH_3OH saturated with HCl, $(CH_3)_2SO_4$ in an alkaline medium, and an ethereal solution of CH_2N_2. The values of W were found from expression (1):

$$W_i = C_{eq}\cdot V_i\cdot M_{eq}\cdot m^{-1}, \tag{1}$$

where C_{eq} is the molar concentration of the equivalents of titrant; usually, C_{eq} = 0.0100 M KOH or HCl for direct and reverse protolyometry, respectively; V_i is the volume of KOH or HCl, liters; M_{eq} is the mass of equivalents of the functional group —COOH (45 g/mole) or —OH (15 g/mole); and m is the weight of the sample of PMPs, g.

The values of pK_i were calculated by means of formula (2) or from the Handerson—Hasselbach equation (3):

$$pK_i = pH - \lg\frac{A_A - A}{A - A_{HA}}, \tag{2}$$

$$pK_i = pH - n\lg\frac{\alpha}{1-\alpha}, \tag{3}$$

where A, A_A, and A_{HA} are, respectively, the optical densities of the solution being analyzed and of solutions containing only the ionized and the unionized forms. The wavelength (λ = 380 nm) was selected so that the difference between A_A and A_{HA} was a maximum: α is the degree of titration of the PMPS and n is an empirical factor.

The different methods of determining W_i and pK_i give close or identical results; at a number of measurements n = 3 and a probability P = 0.95, the confidence limits of the interval of error of the mean value amount to ±0.05; under these conditions the time of performing the methods does not exceed 0.5 h.

Characteristics of the carboxylic (pK_1, W_1) and phenolic (pK_2, W_2; pK_3, W_3) functional groups of different forms of PMP:

Form of PMP	pK_1	W_1, %	pK_2	W_2, %	pK_3	W_3, %	**SEC, mmole/g**
H-	4,7	4,7	7,1	0,7	10,3	3,1	4,5
Na-	5.5	5,8	8,1	1.4	10.9	4,8	5,2
NH_4-	5,0	5,5	7.6	1.3	9,6	4.5	4.9

The facts given above show the weak-acid nature of the pigment and agree with ideas on the structure of a PMP as a statistical copolymer of the products of the oxidative degradation of flavan-3,4-diols. On taking into account the hydroxyoxocarboxylic nature of the PMPs, the presence of "acidic" phenolic groups with pK_2 7.1-8.1 must be ascribed to the —I effect of an adjacent oxo group and to the effect of conjugation [1]. The static exchange capacities (SECs) found at pH 11.5 correlate satisfactorily with the total amounts of functional groups. At the same time, the various forms of the PMPs (OH, Na, NH_4) differ both in their amounts of functional groups and in their ionization constants. This presupposes different degrees of their participation in redox and chemosorption processes, which demands an obligatory standardization of the preparations.

The operative methods described can be used for the control of producton and for evaluating the quality of PMPs.

LITERATURE CITED

1. Yu. L. Zherebin, "The melanin pigments and lipids of the grape and grape wines," Author's Abstract of Doctoral Dissertation, Chemical Sciences, Odessa (1985).
2. G. Hilgetag and A. Martini, Weygand/Hilgetag's Preparative Organic Chemistry, Wiley, New York (1972).