ISOFLAVONES OF THE ROOTS OF Lupinus luteus

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In preceding investigations, it was shown [1] that the main phenolic compounds of the roots of Lupinus luteus L. (European yellow lupin) are five substances of flavonoid nature which have been characterized on the basis of a number of indices as isoflavone glycosides. The results of a study of the structure of these compounds by UV spectroscopy and also by color reactions shows that each of these glycosides has a free hydroxy groups in position 5.

In an investigation of the aglycones obtained by the acid hydrolysis of the glycosides, it was found that glycosides B and C (Table 1) contain the same aglycone, which was identified as genistein (4',5,7-trihydroxyisoflavone) [2]. Glycosides B and C were characterized by their bathochromic shifts and the products of acid and enzymatic hydrolysis as genistein 7-O-β-D-glucoside and genistein 7-O-β-D-glucosylglucoside, respectively.

Glycosides D and E also have the same aglycone, the UV spectrum of which (see Table 1), unlike that of genistein, has a shoulder in the 280-290 nm region, which gave grounds for regarding it as orobol (3',4',5,7-tetrahydroxyisoflavone) or an orobol derivative [3]. From a consideration of the UV spectra with diagnostic additives, it can be seen that the substance contains hydroxy groups in positions 5 and 7, but no shift of the maximum was found with sodium acetate and boric acid. The alkaline degradation of the

TABLE 1. Chromatographic and Spectral Characteristics of the Isoflavones of the Roots of Lupinus luteus L.

Substance	Value of $R_f \times 100$ in systems		Color of the complex with DSA	UV absorption maxima, methanol, nm				
	1	2		neutral solution	KOH	$Zr(OCl)_2$	CH_3COONa	$H_3BO_3 + CH_3COONa$
A genistein C-monoglucoside (A)	0,64	0,54	Brown	264 335	282 320	280 385	280 345	264 335
Genistein 7-O-β-D-glucoside (E)	0,64	0,41	Yellow-orange	262 325	270 355	275 310 380	262 325	262 325
Genistein 7-O-β-D-glucosylglucoside (C)	0,31	0,63	Yellow	259 320	261 345	275 380	259 320	259 320
3',4'-Methylenedioxyorobol 7-O-β-D-glucoside (D)	0,56	0,55	Dark orange	260 285 330	268 360	275 305 380	260 285 330	260 285 330
3',4'-Methylenedioxyorobol 7-O-β-D-glucosylglucoside (E)	0,28	0,70	Orange	258 285 330	261 350	275 380	258 285 330	258 285 330
3',4'-Methylenedioxyorobol	0,89	0,38*	Orange	260 285 335	275 325	275 327 390	269 345	260 285 335
Genistein	0,91	0,26*	Orange yellow	262 325	275 320	270 315 375	272 325	262 325

*System 2 for the aglycones—15% acetic acid.

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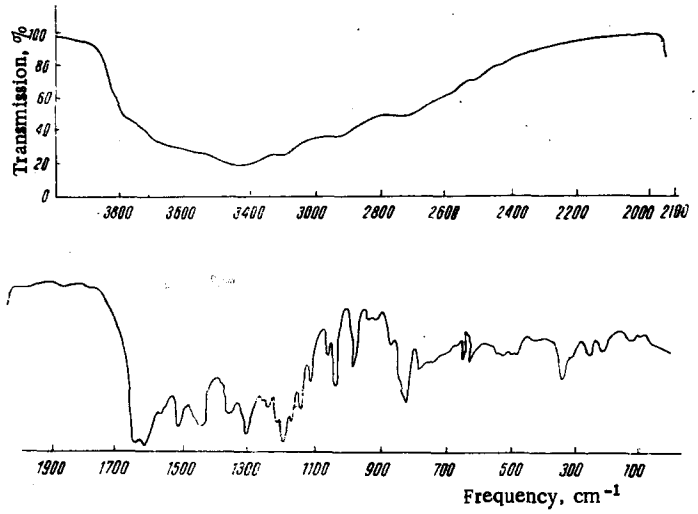

Fig. 1. IR spectrum (KBr) of 5,7-dihydroxy-3'4'-methylenedioxyisoflavone (3',4'-methylenedioxyorobol).

aglycone led to phloroglucinol, 3,4-methylenedioxybenzoic (piperonylic) acid and 3,4-methylenedioxyphenylacetic (homopiperonylic) acid, which were identified by their chromatographic behavior, UV spectra, and color reactions [4-6], the most informative of which is the brick-red coloration on chromatograms with $FeCl_3$. Consequently, the aglycone of glycosides D and E has a 3',4'-methylenedioxy group. The presence of a methylenedioxy group in the molecule of the aglycone is confirmed by the formation of a green coloration on reaction with H_2SO_4 and gallic acid [7], and also by the bands in the IR spectrum characteristic for such groupings at 2950, 2780, 1480, 1400, 1360, 1040, 926, and 800 cm^{-1} [8, 9] (Fig. 1).

Substance A did not give the aglycone on enzymatic hydrolysis and acid hydrolysis with 5% HCl even for 8 h. The shift of the maximum in the UV spectrum of the substance with additives showed the presence in it of three hydroxy groups in positions 4', 5, and 7. Acid hydrolysis by Kiliani's method and reduction of the glycoside with hydriodic acid led to the liberation of genistein. The aqueous residue was found by paper chromatography with markers to contain D-glucose and a small amount of L-arabinose. The same sugars were obtained by the oxidation of the glycoside with ferric chloride. The weight ratio of sugar and aglycone (60 : 40) characterizes substance A as a monoglucoside. What has been said above permits the conclusion that this substance is a genistein C-monoglucoside.

Substance D. A free hydroxy group was found spectrophotometrically only in position 5 (see Table 1). There was no displacement of the maximum with sodium acetate. On acid and enzymatic hydrolysis the glycoside yielded 3',4'-methylenedioxyorobol and D-glucose in a ratio of 50 : 30. Consequently, glycoside D is 3',4'-methylenedioxyorobol 7-O-β-D-glucoside.

Substance E. Analysis of the bathochromic shifts of the UV spectrum showed the presence of a free hydroxy group in position 5. The hydroxy group in position 7 is substituted. Acid and enzymatic hydrolysis gave 3',4'-methylenedioxyorobol and D-glucose in a ratio of 50 : 60. This shows that the molecule of the glycoside contains two glucose residues attached to the aglycone in position 7. Thus, substance E can be characterized as 3',4'-methylenedioxyorobol 7-O-β-D-glucosylglucoside.

EXPERIMENTAL

The substances isolated were chromatographed on FN3 paper in the following solvent systems: 1) isobutanol–acetic acid–water (4 : 1 : 5); 2) 5% acetic acid; 3) butan-1-ol–pyridine–water (6 : 4 : 3); 4) benzene–acetic acid–water (125 : 72 : 3). The UV spectra of the substances were taken on a SF-4A spectrophotometer, and the IR spectra on a UR-20 instrument.

Isolation of the Substances. The isoflavone glycosides were isolated by the preparative chromatography on paper of ethanolic extracts from lupin roots collected in the flowering phase. To obtain the aglycones aqueous extracts from the roots previously purified and evaporated to small volume were hydrolyzed with 5% HCl. The aglycones were extracted with diethyl ether and separated on a column of polyamide (d = 4 cm, h = 80 cm). Elution was performed with a mixture of chloroform and acetic acid (3 : 2).

Alkaline Cleavage of the Aglycones. A solution of 25 mg of one of the aglycones in 20 ml of 25% aqueous NaOH was heated in a sealed tube in the boiling water bath for 2 h. After neutralization, the cleavage products were extracted with ethyl acetate and chromatographed in systems 2 and 4.

Acid Hydrolysis. A solution of 10 mg of one of the glycosides in 5 ml of 5% aqueous HCl was heated in the water bath for 2 h. On hydrolysis by Kiliani's method, the glycoside was dissolved in 5 ml of a mixture of 35 parts of acetic acid, 35 parts of water, and 10 parts of hydrochloric acid. The mixture was heated in the boiling water bath for 6 h. After hydrolysis, the aglycones were extracted with ethyl acetate and chromatographed in system 4. The aqueous residue was neutralized on a column of KU-2 ion-exchange resin and the sugars were chromatographed in system 3.

Enzymatic Hydrolysis. A preparation of the enzyme β-glucosidase obtained from apricot seeds was added to a solution of 5-10 mg of a glycoside in 3 ml of water, and the mixture was placed in a thermostat at 37°C for 24 h. The aglycones were detected by chromatography in system 4, and the sugars in system 3.

Oxidation of the Genistein C-Monoglucoside. A tenfold amount of $FeCl_3$ was added to a solution of 30 mg of the glucoside in 1 ml of water and the mixture was boiled in a sealed tube in the water bath for 6-10 h. Then it was cooled and was brought to pH 8 with an aqueous solution of NaOH, and the precipitate that had deposited was filtered off. The aqueous solution was acidified with hydrochloric acid, purified by the addition of anion- and cation-exchange resins, and dried, and the sugars were chromatographed in system 3.

Reduction of the Genistein C-Monoglucoside. With a 6-fold amount of phenol, 5-10 mg of the glucoside was dissolved in 1 ml of hydriodic acid. The mixture was heated in a sealed tube on an electric hot plate for 6 h. After cooling, a small amount of 20% aqueous sodium bisulfite was added to the mixture. The resinous residue that formed was separated off, washed with water, and dissolved in methanol. The aglycone was studied by chromatography in system 4.

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CONCLUSIONS

For the first time in the isoflavone series, a compound which has the structure of 5,7-dihydroxy-3',4'-methylenedioxyisoflavone (3',4'-methylenedioxyorobol) and two of its glucosides – 3',4'-methylenedioxyorobol 7-O-β-D-glucoside and 3',4'-methylenedioxyorobol 7-O-β-D-glucosylglucoside – and also a genistein C-monoglucoside, have been described. The isoflavones were isolated from the roots of Lupinus luteus L. by preparative chromatography on paper and on columns of polyamide. In addition, the previously known genistein, genistein 7-O-β-D-glucoside (genistin), and genistein 7-O-β-D-glucosylglucoside have been obtained.

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